A Biochemical and Morphological Study of Rat Liver Microsomes

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ABSTRACT

Microsomes isolated by differential centrifugation from a rat liver homogenate in 0.88 M sucrose solution have been studied from the biochemical and morphological point of view.

1. Under these experimental conditions, the “total microsome” fraction was obtained by centrifuging the cytoplasmic extract free of nuclei and mitochondria, for 3 hours at 145,000 g. Morphologically, the total microsomes consist mainly of “rough-surfaced membranes” and “smooth” ones.

2. The total microsomes have been divided into 2 subfractions so that the 1st microsomal fraction contains the “rough” vesicles (2 hours centrifugation at 40,000 g) while the 2nd microsomal fraction consists essentially of “smooth” vesicles, free particles, and ferritin (centrifugation of the supernatant at 145,000 g for 3 hours).

3. By the action of 0.4 per cent sodium deoxycholate in 0.88 M sucrose, it was possible to obtain a pellet for each of the 2 fractions which consisted of dense particles, rich in RNA, poor in lipids, and which represented about 50 to 60 per cent of the RNA and 10 to 15 per cent of the proteins.

The results have been discussed taking into consideration the hypothesis of the presence of RNA in the membranes of microsomal vesicles.

INTRODUCTION

Since the publications of Claude (1–4), then of Hogeboom, Schneider et al. (5–8), of Barnum and Huseby (9), Ada (10), etc., it has been known that the microsomes, submicroscopic particles isolated by differential centrifugation from a homogenate of tissue, differ biochemically from all other cellular fractions by their richness in phospholipids and ribonucleic acid.

More recently, the electron microscope has provided the means for relating the structural elements seen in isolated microsomal pellets to those seen in the intact liver cell (11–14). Thus the examination of ultrathin sections made from a sediment of microsomes reveals essentially two kinds of structures: (a) Profiles of vesicles bearing dense particles of a diameter of 15 mµ attached to the outer surface of the membrane. These vesicles correspond to a cytoplasmic component called ergastoplasm by Dalton et al. (15), Oberling et al. (16–19), and Weiss (20); “rough-surfaced membranes” of the endoplasmic reticulum by Palade and Porter (21–24); α-cytomembranes by Sjöstrand (25, 26). (b) Vesicular formations free of dense particles: they are the “smooth-surfaced membranes” of the endoplasmic reticulum (21, 23, 24). Palade and Siekevitz think that the ribonucleic acid is associated with the dense particles of 15 mµ while the majority of the proteins and nearly all of the phospholipids would belong to the membranes or to the contents of the vesicles (14). The primary importance of these dense particles in the pathways of the protein synthesis has been demonstrated (27).

It is well known that there is a certain biochemical heterogeneity within the microsomal fraction itself: thus the total microsome fraction of rat liver contains several populations of particles, each having different enzymatic properties, but each containing ribonucleic acid (13, 29–32). Moreover, the extraction of the microsomes with
saline solutions has made it possible to obtain fractions which differ one from another in their composition as well as in their metabolic activity (33). In the latter case, the morphologic character of these subfractions was not investigated; it would, however, have been somewhat difficult because of the type of procedure used.

We have studied the microsomes of rat liver by considering in parallel their biochemical composition and their morphological appearance. We have divided the microsomal particles into fractions while trying to retain structural integrity within each of the subfractions. Using differential centrifugation, we were able to obtain 2 definite fractions, one containing all the "rough-surfaced" membranes and the other consisting mainly of "smooth" ones. The phospholipid and ribonucleic acid composition of these subfractions as well as their behaviour with sodium deoxycholate are reported.

**Experimental**

**Animals.**—Male Wistar rats (Commentry and Gif strains) weighing from 230 to 300 gm. were fed on the standard diet of Le Breton (34). The animals were regularly fasted 15 to 18 hours before being killed by decapitation. The liver was promptly removed and a fragment fixed with osmium tetroxide for study with the electron microscope; the quantity necessary for the experiment was weighed, making certain that, if the whole organ was not used, every lobe was represented in the sample.

**Cell Fractionation.**—All the manipulations were made at 4°C. The liver was homogenized with a homogenizer of the modified Potter type (35) in 0.88 M sucrose, so as to obtain a 10 per cent homogenate (w/v). Centrifugations were carried out in the International PRI and Spinco (model L) centrifuges, the centrifugal force being calculated for the bottom of the tube.

The homogenate was centrifuged at 24,000 g for 20 minutes to sediment mitochondria, nuclei, intact cells, and connective tissue. The supernatant was removed by means of a pipette with automatic aspiration. It is from this supernatant that the microsomal fractions were obtained by centrifuging, as indicated in the text. Immediately after the centrifuge was stopped, the non-sedimented phase was removed. The sediment was fixed with osmium tetroxide for examination with the electron microscope. The samples for biochemical analysis were stored at -40°C.

**Conditions for Use of Sodium Deoxycholate (DOC).**—A solution of 0.4 per cent DOC in 0.88 M sucrose adjusted to pH 7.5-7.6 was used. The pellets of the various fractions of microsomes were resuspended in the solution of DOC so that the quantity of DOC equaled at least ½ that of the total proteins of the sediment treated, as indicated by Littlefield et al. (27). Homogenization was carried out very rapidly and the suspensions became clear immediately. After centrifuging 4 hours at 145,000 g (40,000 r.p.m., rotor 40, Spinco model L), a pellet P and a supernatant S were obtained. In some cases, the pellet P was treated a second time with DOC under the same conditions.

Fixation of the pellet P with osmium tetroxide was done immediately after the removal of the supernatant. The sediments obtained (P) and a sample of the fraction of microsomes not treated by DOC, used as a control, were submitted to biochemical analysis.

**Biochemical Methods.**—The samples were analyzed according to the technique of Schmidt-Thannhauser (36), modified by substituting perchloric acid for trichloracetic acid (37). The phospholipids were estimated by the phosphorus of the lipid fraction (Lipid phosphorus = PL.P). Ribonucleic acid was determined with the orcinol reaction (38) from a standard curve established on a pure yeast ribonucleic acid. This result is expressed in ribonucleic acid phosphorus (RNA.P); this value is always smaller than total phosphorus determined on the ribonucleic acid extracts. The difference corresponds to the phosphorus of the protein-bound phosphates which were liberated during the alkaline hydrolysis. The contribution of protein phosphorus to the total phosphorus of the RNA extract varies according to which cellular structures are analyzed (39).

The determination of nitrogen (N) was made on the alkaline digest after extraction of the acid-soluble and lipid fractions: this value thus corresponds to the sum (protein nitrogen + nucleic acid nitrogen). The total phosphorus was determined according to the method of Macherey and Delsal, after sulfuric acid digestion (40).

**Morphological Methods.**—The various samples were fixed in a 2 per cent osmium tetroxide solution buffered at pH 7.3 (41). Sixty minutes later, they were washed with distilled water, dehydrated, then imbedded in n-butyl methacrylate. The ultrathin sections were made with the Servall microtome of Porter and Blunn (42) and observed with the RCA microscope EMU-3B. Six blocks were taken for each sediment fixed; two series of sections were cut from each block at different levels and a large number of examinations made so as to obtain an over-all image of the sediment.

**RESULTS AND INTERPRETATION**

**Total Microsomes**

(a) Isolation of Total Microsomes:

The centrifugal forces used to carry out the sedimentation of microsomes have increased as the centrifuges have become more powerful so that, with 0.88 M sucrose as suspending medium, the earlier centrifugation of 2 hours at 40,000 g
(5, 43) has been changed to 130,000 g (44, 45) and 145,000 g for varying times (14, 46, 47). Except for the reports of Chauveau (30) and of Palade and Siekevitz (14), no rational explanation has ever been given to justify the experimental conditions.

Our results show that from a cytoplasmic extract freed of nuclei and mitochondria, all the particles that sediment up to 3 hours at 145,000 g fit the biochemical definition of the microsomes: that is richness in RNA.P and PL.P. If centrifugation at this speed is carried beyond 3 hours, one observes a drop in the RNA and PL.P (Table I). The decrease first becomes evident with the lipid phosphorus: the sediment obtained between 3 and 4 hours at 145,000 g has a ratio of µg PL.P/mg N which is only about 20 while for the microsomes 100 to 110 is usually found; a decrease of the concentration of RNA.P is also observed (Table I, Experiments 37, 19).

Subsequent fractions (between 4 and 6 hours, and then 6 and 8 hours at 145,000 g) are extremely poor in RNA.P and PL.P (Experiments 37, 17). We have even extended the centrifugation up to 12 hours at 145,000 g in 0.30 M sucrose to see if one could obtain macromolecular particles that might have a special composition. With such conditions, we have obtained a sediment made up principally of proteins (90 per cent of the dry weight—Experiment 31 a). Similar results have also been reported by Palade and Siekevitz: in 0.88 M sucrose, after separation of the microsomes, a centrifugation of 15 hours at 145,000 g gave them a pellet very poor in RNA.P and PL.P (14).

The final supernatant, although very rich in proteins, always contains, nevertheless, a small quantity of non-sedimentable phospholipids and ribonucleic acid. It has been shown that this RNA represents an essential intermediate in protein synthesis (48).

The sediment obtained in 3 hours at 145,000 g represents for us, the "total microsomes." If the sedimented material is assumed to have a mean density of 1.2, calculations indicate that it should contain all particles having a diameter equal to or greater than 60 µm. It is obvious that other smaller elements having a density greater than 1.2 may also be found in the sediment. These conditions agree with the results of Chauveau (30) obtained by granulometric analysis of a homogenate of rat liver. A study of the sedimentation curves as a function of time has permitted him to establish that the whole of the microsomal population could be obtained by a total centrifugation of 165 minutes at 145,000 g in 0.88 M sucrose.

Using the same suspending medium, Palade and Siekevitz find that the lower limit for the microsome fraction corresponds to a centrifugation of 1 hour at 145,000 g; indeed, the sediment that they obtain between 1 and 3 hours at that same speed, shows a drop of 30 per cent in the concentration of ribonucleic acid and of 75 per cent in that of the lipid phosphorus (14). In our experimental conditions, it is possible to extend up to 3 hours the time of centrifugation at 145,000 g and still to obtain microsomal components rich in RNA.P and PL.P.

(b) Composition of the Total Microsomes:

The results obtained for 13 preparations of total microsomes show that their average composition is as follows:

- µg RNA.P per mg. N = 65.1, standard deviation: 5.9
- µg. PL.P per mg. N = 111.5, standard deviation: 9.4

The values found for the concentration in RNA.P are in good agreement with those published by Hogeboom et al. (5), Novikoff et al. (29), Laird et al. (45), but they are definitely smaller than those given by Palade and Siekevitz (14). For the lipid phosphorus, our figures correspond with those of Laird (45), but are, again, lower than those of Palade and Siekevitz (14). One of the reasons for this discrepancy must reside in the fact that the results obtained by the latter authors refer to protein nitrogen, whereas in our experiments nitrogen estimations include both the protein nitrogen plus the nucleic acid nitrogen.

It must be noted that differences in composition are commonly found from one preparation to the other (Tables I and III). Thus, the extreme values

1 A centrifugal field of 145,000 g for the bottom of the tube corresponds to 105,000 g for the middle (Spinco model L, rotor 40).

2 When we centrifuge the cytoplasmic extract for 4 hours at 145,000 g, we obtain a sediment that has practically the same composition as the one obtained after 3 hours at the same speed. This is explained by the fact that since the fraction, which sediments between 3 and 4 hours, is quantitatively very small (in comparison with the material of the total microsomes (see Table II), its compositional differences have little effect on the total constitution of the whole sediment (Table I—Experiments 17, 31).
## TABLE I

RNA and Lipid Phosphorus Content of Microsomal, Postmicrosomal, and Final Supernatant Fractions

(RNA.P in μg.—PL.P in μg.—N in mg.)

<table>
<thead>
<tr>
<th>Experiment Number</th>
<th>37</th>
<th>19</th>
<th>17</th>
<th>31 a</th>
<th>32 b</th>
<th>32</th>
<th>14</th>
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<tr>
<td></td>
<td>PL.P</td>
<td>RNA.P</td>
<td>PL.P</td>
<td>RNA.P</td>
<td>PL.P</td>
<td>RNA.P</td>
<td>PL.P</td>
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<tr>
<td>Microsomal subfractions</td>
<td>108.5</td>
<td>60.3</td>
<td>113.5</td>
<td>66.7</td>
<td>130</td>
<td>61.3</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>89</td>
<td>72.7</td>
<td></td>
<td></td>
<td>110</td>
<td>65.1</td>
<td>121</td>
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<tr>
<td></td>
<td>99.5</td>
<td>78.4</td>
<td></td>
<td></td>
<td>122</td>
<td>71.4</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>78.4</td>
<td>68</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postmicrosomal fractions</td>
<td>17.3</td>
<td>49.2</td>
<td>20.7</td>
<td>53.8$</td>
<td>111$</td>
<td>62.5$</td>
<td>101.5$</td>
</tr>
<tr>
<td></td>
<td>10.5</td>
<td>18.9</td>
<td></td>
<td></td>
<td>3.1</td>
<td>6.9</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>16.9</td>
<td>4.5</td>
<td>13.1</td>
<td>4.2</td>
<td>3.9</td>
<td>4.4</td>
<td>12.2</td>
</tr>
<tr>
<td>Supernatant‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Nuclei and mitochondria are removed

Each fraction is obtained by centrifuging the supernatant of the preceding centrifugation: for instance, in Experiment 37, the homogenate freed of nuclei and mitochondria is centrifuged at 40,000 g for 20 minutes. The sediment is called “microsomal subfraction 20 minutes at 40,000 g”; after decantation, the supernatant is centrifuged at 40,000 g for 2 hours in order to obtain a sediment (referred to ‘microsomal subfraction 2 hours at 40,000 g’) and a supernatant. This is then centrifuged at 145,000 g for 1 hour... etc. The same procedure is applied to all the experiments.

* Centrifugation made in 0.3 M sucrose.
‡ Supernatant of the last centrifugation.
§ These 2 sediments contain all the microsomes.
TABLE II
Distribution of Nitrogen, PL.P, and RNA.P between the Microsomal and Postmicrosomal Fractions
(Results expressed in µg. per 100 mg. of wet liver)

<table>
<thead>
<tr>
<th>Experiment Number</th>
<th>N</th>
<th>PL.P</th>
<th>RNA.P</th>
<th>N</th>
<th>PL.P</th>
<th>RNA.P</th>
<th>N</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 hours at 40,000 g</td>
<td>226.3</td>
<td>21.9</td>
<td>15.3</td>
<td>254</td>
<td>32.05</td>
<td>17.25</td>
<td>318</td>
<td>36.1</td>
<td>21.2</td>
</tr>
<tr>
<td>1 hour at 145,000 g</td>
<td>118</td>
<td>11.73</td>
<td>9.25</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 hours at 145,000 g</td>
<td>84.2</td>
<td>6.55</td>
<td>9.64</td>
<td>133</td>
<td>19.55</td>
<td>9.64</td>
<td>144</td>
<td>15</td>
<td>9.8</td>
</tr>
<tr>
<td>Postmicrosomal fractions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>4 hours at 145,000 g</td>
<td>15.6</td>
<td>0.27</td>
<td>0.63</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>6 hours at 145,000 g</td>
<td>13.2</td>
<td>0.14</td>
<td>0.25</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supernatant</td>
<td>675</td>
<td>8.27</td>
<td>4.24</td>
<td>667</td>
<td>11.3</td>
<td>3.04</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For the scheme of centrifugation, see the legend of Table I.

that we obtained for the ratio RNA.P/N were 51.3 and 79.5; however, such are exceptional, in particular the figure 51.3. As a rule, the ratios range between 60 and 68. As for the ratio PL.P/N, the values below 95 and above 125 are infrequent.

The microsomes contain 29 per cent phospholipids, 7 per cent ribonucleic acid, and 64 per cent proteins by dry weight.

As far as the quantitative evaluation of the cell fractions is concerned, it was found that the “total microsomes” correspond to about 420 µg. of the nitrogen and the final supernatant to 670 µg. per 100 mg. of wet liver. The distribution of N, RNA.P, and PL.P among the various microsomal and postmicrosomal fractions obtained for 3 routine experiments is reported in Table II.

(c) Fractionation of the Total Microsomes:

In order to test the biochemical homogeneity of the total microsomes, we have subdivided them into several subfractions arbitrarily determined. Taking into account the normal variations from one experiment to another, the results show that no significant nor really systematic variations exist within a single preparation in regard to the ratios RNA.P/N and PL.P/N (Table I); in particular, one cannot demonstrate a gradient of concentration in ribonucleic acid as a function of the size of the microsomal structures.

(d) Morphology:

(1) Total Microsomes.—The examination with the electron microscope of suspensions of microsomes placed on a plastic film reveals only non-structured granules of various sizes (49). It was necessary to wait for the introduction of the technique of ultrathin sectioning to bring out the internal structure of the microsomes and thus establish their relationship with the membranous components which exist in the cell in situ.

Examination of a section of a pellet of total microsomes reveals the existence of several elements (Fig. 1):

Vesicular structures, with circular or oval profile, of various sizes having numerous dense particles attached to the outer surface of their membranes (50). The length of the vesicles varies according to their main axis, from about 80 to 400 µm. We know that these formations are the result of the fragmentation of the ergastoplasm (11) or, according to the terminology of Palade and Porter, of the rough surfaced membranes of the endoplasmic reticulum (14). Palade and Siekevitz have shown that the swollen aspect of the vesicles in the sediments of microsomes is due to the hypotonicity of the fixative.

Numerous vesicles with a circular or oval profile, totally free of dense particles, of a length varying according to their main axis, from 70 to 300 µm.
Rat liver microsomes

They are the smooth surfaced membranes of the endoplasmic reticulum.

Free dense particles identical to those attached to the ergastoplamic membranes.

A few dense bodies identical to those described by Palade and Siekevitz (peribiliary bodies) (14) and by Novikoff et al. (51); occasionally, small mitochondria.

Small spheres, extremely dense to the electrons, with sharp outlines, of a diameter of 60 A, which correspond to grains of ferritin (52).

(2) Postmicrosomal Fractions.—Fraction sedimented between 3 and 4 hours at 145,000 g: free particles and vesicles are still present, but zones of amorphous material begin to appear; numerous particles of ferritin (Fig. 2). Fraction sedimented between 4 and 6 hours at 145,000 g: almost complete disappearance of the vesicles, the section consisting mostly of areas of amorphous material; presence of ferritin (Fig. 3). Fraction sedimented between 6 and 8 hours at 145,000 g: the vesicles have disappeared, the preparations consisting entirely of amorphous material.

Microsomal Fractions

The sole aim of the fractionation reported in Table I was to follow the changes in the concentrations of RNA and PLP during centrifugation of the microsomal particles; therefore, no theoretical principle was adopted for the centrifugation procedure.

An interesting basis for subdividing the microsomes into different fractions seems to us to reside in the possibility of separating the “smooth vesicles” from the “rough” ones. This would make it possible not only to establish their respective composition, but also to undertake the study of their metabolic activity. Certainly the presence of dense particles (density about 1.5) attached to the outside of the membranes must confer a greater total density to the rough vesicular formations than that possessed by the smooth ones; moreover, the diameter of structures lacking particles is generally less than that of the other ones. Hence, one should expect the ergastoplasmic vesicles to sediment more rapidly. The centrifugation procedure adopted was the following:

1st microsomal fraction: sediment obtained on centrifuging the nuclei and mitochondria-free homogenate at 40,000 g for 2 hours.

2nd microsomal fraction: sediment obtained on centrifuging the above supernatant at 145,000 g for 3 hours.

The sediments of the 2nd fraction are always dark red and quite translucent while those of the 1st one are of a paler color and more opaque. The 2 sediments show distinct boundaries and we never observe any fluffy material when removing the supernatant.

The biochemical analysis shows that the concentrations of the RNA and PLP per mg. of nitrogen are very similar in the 2 subfractions and that any differences are not significant (Table III). These findings do not call for any comment; they compare well with the results obtained previously (Table I). The quantitative evaluation of the 2 subfractions shows that there is twice as much nitrogen in the
Furthermore, the results obtained by one of us (Y. M.), in collaboration with Dr. E. L. Kuff, seem to prove that the specific activity per mg. of nitrogen of DPNH-cytochrome c reductase, glucose-6-phosphatase, and esterase was of the same order in both fractions (53).

The electron micrographs of the 1st microsomal fraction show a large number of rough surfaced membranes (Fig. 5). Nevertheless, a certain number of smooth forms may also be observed and it is impossible to avoid their presence if one wants to obtain a sediment containing all the membranes bearing particles, including the small sized ones. However, it is possible to obtain an extremely pure ergastoplasmic fraction by centrifuging the cytoplasmic extract free of mitochondria for only 20 minutes at 40,000 g; this fraction then only contains very few large sized smooth membranes (Fig. 4).

It is obvious that morphological structures other than those resulting solely from the fragmentation of the endoplasmic reticulum must also be centrifuged down in this fraction. Thus small mitochondria may be found, and a few peribiliary bodies. Moreover, uricase assays show that the specific activity of this enzyme is of the same order in the 1st microsomal fraction as in isolated mitochondria, whereas in the ergastoplasmic fraction obtained after centrifuging for 20 minutes at 40,000 g, it was more than twice as high; this enzyme is absent in the 2nd microsomal fraction and in the final supernatant. Thus the morphological structures bearing the uricase activity accompany the sedimentation of the rough surfaced membranes (54).

The electron micrographs of the 2nd microsomal fraction show numerous smooth vesicles, some of which are very small; the rough profiles appear only occasionally (Fig. 7). Moreover, free dense particles (10 to 15 m in diameter) and ferritin are also found. It is known that in the liver cell, the free particles are much less numerous than in the pancreatic exocrine cell (55). Under the present experimental conditions, the sedimentation of structural elements of this size may at first appear surprising; but their high density (about 1.5 for the free particles and 1.8 for the ferritin) may account for their presence in the sediments.

In most cases few free dense particles were observed in the ultrathin sections (Fig. 7); however, in some preparations we found a large quantity of these free particles forming dense patches between the vesicles (Fig. 9); it should be mentioned that the RNA.P/N ratios for these sediments were always at the upper limits of the range of values usually obtained for the 2nd microsomal fraction (Table I: Experiment 32; Table IV: Experiment 29 b).

It has been shown that the smooth membranes of the endoplasmic reticulum give rise to smooth vesicles after fragmentation; but the possibility that other morphological structures of liver tissue may also be present in the homogenate in the form of membranes lacking particles cannot altogether be excluded. This might be the case for biliary microvilli (56) and for Golgi membranes. The centrifugation procedure which has been devised for obtaining the 2nd microsomal fraction does not permit the separation of these various structures.

Action of the Sodium Deoxycholate on Microsomes

The bile salts, with their surface-active properties, are often used to break some bonds existing in complex biological molecules; Littlefield et al. (27), then Palade and Siekevitz treated the isolated microsomes of rat liver with sodium deoxycholate (DOC) (14). After a prolonged centrifugation, the latter authors obtained a small pellet which had a concentration in ribonucleic acid 3 to 8 times larger than that of the initial microsomes, was poor in lipids, and which on morphologic examination was shown to be composed of granular particles. Their interpretation was that these granules correspond to the small dense particles of the rough surfaced membranes, which were freed as a consequence of the action of deoxycholate in rupturing the vesicles and solubilizing the membranes.

The possibility of obtaining smooth vesicles and rough ones separately, enables one to study the behaviour of the 2 kinds of membranes after treatment with DOC. We treated the 2 microsomal fractions with the detergent under the conditions mentioned in the experimental section. The RNA.P and PL.P concentrations, and the morphological characteristics of these sediments are reported in Table IV.

The addition of DOC to a suspension of microsomes immediately clarifies the solution. After centrifuging for 4 hours at 145,000 g, the final pellets P are cohesive and well packed; the pale yellow supernatant is quite clear; in 0.88 m sucrose solution, there was no incompletely sedimented material (fluffy material) above the sediment, as had been observed when using 0.25 m sucrose or
TABLE IV
Biochemical and Morphological Composition of the Microsomal Subfractions before and after the Treatment with Sodium Deoxycholate

<table>
<thead>
<tr>
<th>1st microsomal fraction</th>
<th>Morphology</th>
<th>mg. PL.P</th>
<th>mg. RNA.P</th>
<th>RNA.P</th>
<th>Nitrogen</th>
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<tr>
<td>Preparation 26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Majority of rough vesicles</td>
<td>120.5</td>
<td>55.7</td>
<td>(100)</td>
<td>(100)</td>
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<tr>
<td>Final pellet</td>
<td>Dense granular elements</td>
<td>1.9</td>
<td>296</td>
<td>54</td>
<td>10</td>
</tr>
<tr>
<td>Preparation 29</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control (Fig. 5)</td>
<td>Majority of rough vesicles</td>
<td>128</td>
<td>60.8</td>
<td>(100)</td>
<td>(100)</td>
</tr>
<tr>
<td>Final pellet (Fig. 6)</td>
<td>Dense granular elements</td>
<td>4.3</td>
<td>288</td>
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<table>
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<th>2nd microsomal fraction</th>
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<th>mg. RNA.P</th>
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</tr>
<tr>
<td>Control</td>
<td>Many smooth vesicles</td>
<td>94</td>
<td>64</td>
<td>(100)</td>
<td>(100)</td>
</tr>
<tr>
<td>Final pellet</td>
<td>Dense granular elements</td>
<td>1.8</td>
<td>208</td>
<td>51.6</td>
<td>15.9</td>
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<tr>
<td>Preparation 34</td>
<td></td>
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<td></td>
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<tr>
<td>Control (Fig. 7)</td>
<td>Many smooth vesicles</td>
<td>127</td>
<td>63</td>
<td>(100)</td>
<td>(100)</td>
</tr>
<tr>
<td>Final pellet (Fig. 8)</td>
<td>Dense granular elements</td>
<td>4.3</td>
<td>239</td>
<td>40</td>
<td>10.5</td>
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<tr>
<td>Preparation 29 b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (Fig. 9)</td>
<td>Smooth vesicles and many free particles</td>
<td>—</td>
<td>73.6</td>
<td>(100)</td>
<td>(100)</td>
</tr>
<tr>
<td>Final pellet (Fig. 10)</td>
<td>Dense granular elements</td>
<td>1.8</td>
<td>254</td>
<td>53</td>
<td>15.3</td>
</tr>
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From the results reported in Table IV it is clear that:

1. The final pellets P obtained after treatment with DOC all show high ribonucleic acid concentration; the RNA.P/N ratio is 4 to 5 times greater than that of non-treated sediments (from 208 to 296 instead of from 55.7 to 73.6). The absolute value of this ratio is lower than those obtained by other authors, but under our experimental conditions, the nitrogen determinations correspond to the estimation of protein nitrogen plus nucleic acid nitrogen. A calculation of the RNA.P/protein N ratio from our experimental figures gives values ranging between 320 to 595; thus they are of the same order as those obtained by Palade and Siekevitz (14). It may be presumed, then, that the structures which have been isolated contain 35 to 45 per cent RNA when related to dry weight. These results agree with those obtained by Petermann and Hamilton during purification of isolated RNP particles from rat liver microsomes treated by DOC (57).

2. These pellets all are extremely poor in phospholipids: PL.P/N = 1.8 to 4.3 instead of about 110 for the untreated pellet.

3. From the morphological point of view, the pellets consist of dense, granular elements which are very similar to the dense particles visible in the sections of microsomes before the DOC treatment (Figs. 6, 8, 10).

4. The pellets P represent 40 to 64 per cent of the ribonucleic acid and 10 to 15 per cent of the nitrogen of the initial sediments. For the same suspension medium and for similar DOC concentrations, the RNA recovery reported by Palade and Siekevitz amounted to 80 to 90 per cent. We have never been able to obtain these RNA percentages in our sediments under similar experimental conditions. It should be mentioned that the freezing of the sediments before the biochemical analysis is not responsible for this situation: two samples—one frozen, the other one unfrozen—give the same percentage of sedimented RNA (Table V). Similarly, the instability of the RNA particles in the absence of Mg++, cannot be invoked as the cause of this discrepancy (see 28 for references), since the use of a 0.4 per cent solution of DOC containing 0.0005 M Cl2Mg also results in the sedimentation of only 50 per cent of the initial RNA (53). The partial solubilization of the microsomal RNA after DOC treatment has already been observed in tissues other than the liver. The pancreatic microsomes treated by DOC at a final concentration of 0.3 per cent produced, after centrifugation, a pellet of par-
Y. MOULÉ, C. ROUILLER, AND J. CHAUVEAU

TABLE V
Effect of Freezing on the Microsomes before the DOC Treatment

<table>
<thead>
<tr>
<th>DOC treatment after freezing of the microsomes</th>
<th>Final pellet</th>
<th>Supernatant</th>
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</thead>
<tbody>
<tr>
<td>DOC treatment with-freezing of the microsomes</td>
<td>Final pellet</td>
<td>Supernatant</td>
</tr>
</tbody>
</table>
| particles which represented only about 50 per cent of the initial RNA (55). Furthermore, it is quite possible that DOC treatment may bring about a certain “labilization” of microsomal components, especially proteins; the total recovery of the RNA, PL.P, and proteins is sometimes lower than that usually obtained without DOC. An ordinary cellular isolation yields a total recovery of 92 to 100 per cent; when DOC is used, this value may range between 80 to 85 per cent.

It may be concluded that whatever the microsomal fraction, whether it is composed of a majority of rough vesicles or of a majority of smooth ones, treatment with sodium deoxycholate enables one to obtain a pellet consisting of dense particles, poor in PL.P, rich in RNA, and which represent about 50 per cent of the initial RNA.

These findings will be discussed.

DISCUSSION

The “total microsome” fraction defined by our experimental results extends for the 0.88 M sucrose medium between 20 minutes at 24,000 g and 3 hours at 145,000 g. It is evident that the sediment thus obtained does not include all the microsomes present in the homogenate; the largest constitute a part of the “fluffy layer” which tops the mitochondrial fraction; the smallest complete their sedimentation between 3 and 4 hours at 145,000 g and beyond. But the conditions adopted define an area of biochemical homogeneity with regard to the concentration of ribonucleic acid and phospholipids; outside of this zone all fractions have always a smaller RNA:P and PL:P per mg. nitrogen ratio. Thus, the term “microsome” corresponds to a certain type of biochemical composition; it still conforms to the definition given by Claude who considered these structures as “phospholipide-ribonucleoprotein complexes” (1, 2). We must point out that this biochemical homogeneity concerns only the ribonucleic acid and the phospholipids, because it is known that within this group there are localizations of enzymatic activities which are a function of the sizes of the components (15, 31).

The fractionation of the “total microsomes” in several subfractions demonstrates the fact that all the microsomal structures have comparable amounts of RNA:P and PL:P whatever their size. These results are opposed to those previously found by several authors who noticed that the smaller the diameter of the microsomes, the richer they are in ribonucleic acid (9, 52, 58). It must be noted that the increase of concentration in RNA as an inverse function of the size of the microsomal structures was observed only with a saline media for mouse liver (9, 58) or in 0.25 M sucrose for rat liver (52).

Since the delimitation of the microsomes was based on no particular morphological criterion, it was conceivable that their composition might well be somewhat heterogeneous. Indeed, the ultrathin sections of the fixed liver microsomes show that the majority of the structures correspond to the fragmentation of the endoplasmic reticulum (11, 14); they appear mainly in the form of vesicles either with or without dense particles attached to the outside; according to the terminology given by Palade and Porter, these would be the “rough” and the “smooth” surfaced membranes. Similar conclusions have been made for microsomes isolated from other tissues: pancreas, brain (55, 59, 60).

Because of their size and density (the presence of dense particles “adding weight” to the vesicles), the rough elements are the first to sediment; after 20 minutes at 40,000 g, the sediment obtained consists almost entirely of membranes with particles attached to the outside. By prolonging the centrifugation, the number of “smooth” forms gradually increase. We have defined experimental conditions in such a way as to obtain 2 microsomal subfractions, one of which contains almost all the membranes with attached particles, and the other containing membranes free of particles.

On comparing the biochemical with the morpho-
logical results, the presence of RNA in a microsomal fraction does not parallel the frequency of the rough membranes; we can obtain sediments characterized by a majority of smooth vesicles where the concentration of RNA is not significantly different from that in sediments with a majority of rough membranes. The RNA P/N ratios become systematically much lower only when the structural elements disappear, while the amorphous material which is presumably protein in nature increases in amount (postmicrosomal fractions). We know that free particles exist in the 2nd fraction and it may be objected that the RNA that we find is due solely to these particles, and has no connection with the microsomal membranes, as we have previously suggested (61). In most cases however, the micrographs show very few particles, although of course, a representative section of the pellet would provide more convincing evidence. It should also be mentioned that a substantial part of the sediment is always fixed and that a large number of examinations have been made. Moreover, when pellets rich in free particles are found, it is impossible to miss these particles: indeed, they cover the section (Fig. 9), all the blocks having the same appearance. Further physiological investigations are required to elucidate the reason for the presence of an unusually large number of free particles in isolated cases. It seems therefore that the localization of RNA exclusively in the particles may be questioned.

The action of sodium deoxycholate on the microsomes gives very interesting results. As early as 1956 it enabled Palade and Siekevitz to isolate ribonucleoprotein particles containing 30 to 40 per cent RNA (14). The essential role these particles play as intermediates of protein synthesis has since been demonstrated (see 28 and 62). The results we have obtained by the action of DOC on the 2 microsomal fractions, defined above, call for some comment. If one admits that the DOC spares the preexisting dense particles in the microsomes, whether they be free or attached to rough membranes, then one must try to explain the origin of the RNA found present in the DOC supernatant and which corresponds to about 45 per cent of the microsomal RNA. The hypothesis that RNA may be found present in the membranes themselves enables one to understand these facts: the DOC treatment would break the bonds between the macromolecules of the membranes and thus, would lead to the "solubilization" of their components (proteins, phospholipids, RNA). It would be interesting to know the form in which the RNA of the DOC supernatant exists: is it present in the free state or does it remain attached to other molecules, if so, what are the molecules? Some experiments carried out by one of us (Y. M.) in collaboration with Dr. E. L. Kuff (53), and some investigations now in progress in our laboratory tend to show that this RNA forms part of particulate fragments smaller and/or lighter than RNP particles. We need more data in order to elucidate this important problem.

The presence of RNA in the microsomal membranes does not agree with the first results obtained by Palade and Siekevitz (14); however, in a recent publication, Palade stated that it was not possible to limit the presence of RNA exclusively to the dense particles since "the particles do not account for ~20 per cent of the microsomal RNA in the liver and for ~40 per cent in the pancreas" (see 28). Moreover, this opinion would agree with work carried out by Kuff, Hogeboom, and Dalton: using a technique, the principle of which is quite different from ours, the authors came to the conclusion that liver microsomes are distributed in several groups which are distinguishable by their enzymatic activities, but which all contain ribonucleic acid, while the typical ergastoplasmic formations correspond only to one single group (13). The presence of RNA in the microsomal membranes would also agree with the fact that the specific activity of RNA labeled in vivo after injecting P32, was not the same in the RNA of the pellet and of the supernatant obtained by treatment of liver microsomes with DOC (63, 64). All these findings provide further evidence that the microsomal RNA must be considered as metabolically heterogeneous (63, 64, 66).

Up to the present time, as far as we know, the RNA particles of the liver have only been isolated after treatment with DOC. From the biochemical and physiological point of view, it would be interesting to separate in the 2nd microsomal fraction, the free dense particles from the smooth surfaced membranes; this would also enable one to see to what extent DOC treatment modifies their com-

4 For the pancreas, after injecting adenine-8-C14, Siekevitz and Palade reported specific activities of RNA which are different for the attached RNP and for the "microsomal contents" (65).

5 In the case of the pancreas, Palade and Siekevitz succeeded in isolating the free particles (55). Similar results have also been obtained from plants and bacteria (see 28).
position. This investigation is now in progress in our laboratory.

We want to thank Miss C. Bouzonviller and Mrs. A. Donchtell for their efficient technical assistance.

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EXPLANATION OF PLATES

List of Abbreviations

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>t</td>
<td>0.5 μ</td>
</tr>
<tr>
<td>b</td>
<td>dense bodies</td>
</tr>
<tr>
<td>f</td>
<td>particles of ferritin</td>
</tr>
<tr>
<td>M</td>
<td>mitochondria</td>
</tr>
<tr>
<td>p</td>
<td>dense particles described by Palade</td>
</tr>
<tr>
<td>r</td>
<td>rough surfaced membranes or vesicles</td>
</tr>
<tr>
<td>s</td>
<td>smooth surfaced membranes or vesicles</td>
</tr>
</tbody>
</table>

PLATE 295

Fig. 1. Ultrathin section of a pellet of total microsomes obtained by 3 hours centrifugation at 145,000 g. Note the different structures of the hepatic microsomes.

1. Rough surfaced vesicles characterized by numerous dense particles of a diameter of 10 to 15 nm attached to the outer surface of their membrane.
2. Smooth surfaced vesicles which do not bear dense particles. Both these elements make up the greatest part of the sediment. Vesicles often contain a fine precipitate whose density is below that of the membranes.
3. A few small mitochondria.
4. Dense bodies.
5. A few small, very dense particles of a diameter of about 60 Å, corresponding to the ferritin.

Magnification: × 72,000.

Fig. 2. Section of pellet of a postmicrosomal fraction sedimented between 3 and 4 hours at 145,000 g. Large number of small smooth surfaced vesicles whose profile is masked by the presence of amorphous material; dense grains of ferritin. Magnification: × 72,000.

Fig. 3. Section of pellet of a postmicrosomal fraction sedimented between 4 and 6 hours at 145,000 g. The few rare vesicles and the dense particles are difficult to bring out because of the presence of amorphous material, much more abundant than in the preceding subfraction (Fig. 2). Numerous small grains, very dense, probably corresponding to the grains of ferritin. Magnification: × 72,000.
PLATE 296

Fig. 4. Section of pellet obtained by 20 minutes centrifugation at 40,000 g. Note the majority of "rough surfaced membranes." Magnification: × 73,000.
(Moulé et al.: Rat liver microsomes)
Fig. 5. Section of pellet of the 1st microsomal subfraction obtained by 2 hours centrifugation at 40,000 g: Preparation Number 29. Many rough surfaced membranes and few smooth surfaced vesicles. Magnification: X 73,000.

Fig. 6. Section of pellet P 29 obtained by 4 hours centrifugation at 145,000 g of 1st microsomal fraction Number 29 treated with DOC. This pellet is composed of dense granular elements of a diameter of 10 to 15 μm morphologically identical to those attached to the rough surfaced membranes. Presence of a finely granular material of a lower density than that of the dense granulations and of a few rare small vesicles (arrows). Magnification: X 73,000.
Fig. 7. Section of pellet of the 2nd microsomal subfraction sedimented between 2 hours at 40,000 g and 3 hours at 145,000 g; preparation Number 34. Larger number of smooth surfaced vesicles and a few dense free particles. Magnification: X 73,000.

Fig. 8. Section of pellet P 34 obtained by 4 hours centrifugation at 145,000 g of 2nd microsomal fraction Number 34 treated with DOC. This sediment is essentially composed of dense granular elements. Magnification: X 73,000.
Fig. 9. Section of pellet of the 2nd microsomal subfraction: preparation Number 29 b. In this case, predominance of free dense particles; a few smooth surfaced vesicles. Magnification: $\times 55,000$.

Fig. 10. Section of pellet $P$ 29 b obtained by 4 hours centrifugation at 145,000 g of subfraction Number 29 b treated with DOC. This sediment is essentially composed of dense granular elements. Magnification: $\times 73,000$. 
(Moulé et al.: Rat liver microsomes)