LIVER MICROSOMES

An Integrated Morphological and Biochemical Study*

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INTRODUCTION

Following the introduction of suitable techniques for the separation of cell components by differential centrifugation (2, 3), Claude succeeded in isolating from homogenates of liver and other tissues a fraction of "submicroscopic"[1] particles referred to initially as "small granules" (2) and later as "microsomes (4, 5). He also found that the fraction concerned was rich in ribonucleic acid (RNA), a property confirmed in detail by subsequent investigators who showed that the microsomal RNA amounts to ~9 per cent of the microsomal dry weight (6) and accounts for nearly half of the RNA of the whole cell (*cf.*, 7, 8). The finding suggested from the beginning that the microsomes were derived from the so called basophilic substance of the cytoplasm, a view supported by evidence brought forward by Claude (5, 9) and Brenner (10). Since Claude's findings, attempts at more detailed fractionation of tissue homogenates have yielded fractions of particles apparently smaller than microsomes (6, 11–14) characterized by an even higher content of RNA and referred to sometimes as "ultramicrosomes" (6) or "macromolecules of ribonucleoprotein" (12–14).

In recent years, a number of studies have shown that the incorporation of labelled amino acids proceeds at a higher rate in microsome proteins than in the proteins of any other cell fraction both *in vivo* (15–20, 22) and *in vitro* (21–23). This property was taken to suggest that the microsomes are active in protein synthesis and probably represent a cell organelle specialized in this direction.

It must be pointed out that the microsomes, as well as the smaller and related particles, *e.g.* ultramicrosomes and macromolecules, have remained thus far cytochemical concepts, without any known morphological counterpart in the intact cell, because for a long time the study of the morphology of the cell at the corresponding dimensional level lagged behind cytochemical investigation. During the last few years, however, improvements in preparatory techniques for electron microscopy have changed this situation. Electron

* Part of the findings presented in this article have already been published in abstract (1).

[1] By reference to the light microscope.

microscope observations of animal cells have demonstrated that in the dimensional range of the microsomes the cytoplasm possesses a network of membrane-bound vesicles and tubules described as the endoplasmic reticulum (24, 25). Within this system there are differentiated portions in which small, dense particles, 10 to 15 mμ in diameter, appear to be attached to the membrane of the reticulum (26, 27). Although this association is commonly encountered in certain cell types, including parenchymatous liver cells, in many other types the particles mentioned occur freely scattered in the cytoplasmic matrix. A study of their distribution among various cell types has shown that these particles correlate better with cytoplasmic basophilia than does any other known cellular component (26). There is therefore at present considerable information available about the organization of the animal cell in the dimensional range in which microsomes belong and, under such circumstances, an attempt to correlate cytochemical and morphological concepts appears justified. The present paper describes such an attempt and arrives at an interpretation of the cytochemical concept of microsomes in terms of intracellular structures. It shows that the microsomes are derived mainly from the endoplasmic reticulum and that their characteristically high RNA content is due to associated small particles.

EXPERIMENTAL

General Plan of the Experiments

The rat liver was selected as a microsome source because most of the present biochemical information concerning the microsomal fraction has been derived from studies of this material (7, 8). The method using hypertonic sucrose (28) for the homogenization and fractionation of the liver was chosen because it is known to insure a better morphological preservation of cell organelles than methods using either isotonic sucrose or saline.

In each experiment, small fragments of liver tissue were excised and fixed for electron microscopy before homogenizing the rest of the organ. The fate of the various cell components was followed throughout homogenization and fractionation by examining in the electron microscope samples of tissue, homogenate, and fractions derived from the same liver. The liver tissue was examined in sections, whereas the homogenate and the fractions derived therefrom were examined either in suspension or in sections of embedded material. In the case of the microsome fraction, aliquots were taken in each experiment for: (*a*) fixation and subsequent electron microscopy and (*b*) chemical determinations. These latter comprised quantitative measurements of ribonucleic acid (RNA), protein nitrogen (P-N), and phospholipide phosphorus (PLP-P), as well as determinations of more characteristic microsomal components, *e.g.*, the hemochromogen described by Strittmatter and Ball (29,

30) and the enzyme diphosphopyridine nucleotide–cytochrome *c* reductase studied by Hogeboom (31).

Since two morphologically distinct components, *i.e.* a membranous component and a small particulate component, were found associated in the microsome fraction, attempts were made to disassociate them, or to destroy one or the other, by various procedures such as "aging" at various temperatures, treatment with ethylenediaminetetraacetate (versene) or deoxycholate, and incubation with ribonuclease. In each experiment, aliquots of treated microsomes and of controls were secured for electron microscopy as well as for the chemical determination of the components already mentioned.

Homogenization and Fractionation.—For all experiments albino rats of the Sprague-Dawley strain weighing ~250 gm. were used. After being fasted for 16 to 20 hours, in order to minimize the contamination of liver fractions by glycogen, the animals were killed by a blow on the skull and allowed to bleed as much as possible. Small fragments of liver were excised for fixation, while the rest of the organ was pulped by forcing it through a tissue masher. In each experiment 12 to 15 gm. liver pulp, usually pooled from two animals, were homogenized in a small volume of 1.46 M sucrose (1 ml. per 1 gm. liver pulp)[2] and then diluted with 0.88 M sucrose until the homogenate reached a 1 to 10, weight to volume, dilution.

The liver homogenate was centrifuged for 30 minutes at 20,000 *g* in order to sediment, in a single run, tissue debris, whole and broken cells, nuclei, mitochondria, and the "fluffy layer" (33). Although the latter is known to contain a large amount of microsomal material (7), it was left behind in order to minimize the contamination of microsomes by mitochondria and other, apparently different, granules of ~0.1 μ diameter (33). For separating the microsome fraction, the supernatant of the first run, which corresponds to the usual mitochondrial supernatant, was centrifuged for 60 minutes, at 105,000 *g*. The centrifugal force used is higher than that employed in the original procedure (28). In the conditions of these experiments and according to Pickels' formula (34), it should cause the sedimentation of particles larger than 35 mμ within 1 hour. The homogenization and fractionation were carried out at low temperature (0–4°C.) using a glass homogenizer with a motor-driven teflon pestle, precooled equipment, solutions and containers, and a refrigerated centrifuge (Spinco, model L, Specialized Scientific Instruments, Belmont, Calif.) equipped with an angle-head rotor. In order to render directly comparable the data obtained in various fractions, the volume of each fraction was usually brought up to the original volume of the homogenate with 0.88 M sucrose. Thus 1 ml. of microsome fraction, for instance, contained the same amount of microsomes, as 1 ml. of homogenate or 100 mg. wet weight liver pulp. Since the microsomes were centrifuged down in plastic tubes containing 10 ml. mitochondrial supernatant, the pellet obtained in each tube represented microsomes originally present in 1 gm. liver pulp.

Chemistry

Preparations.—After pipetting off the microsomal supernatant, a small amount of 0.88 M sucrose was added to the tubes containing the translucent, tightly packed and red colored microsome pellets. Each pellet was homogenized in its centrifuge tube with a motor-driven glass pestle and the suspension was then made up to a volume of 10 ml. with 0.88 M sucrose. Chemical determinations were always done on two duplicate pellets. In experiments requiring

[2] This procedure results in a high percentage (80 to 90 per cent) of cell breakage (32).

incubation of microsomes under various conditions, or treatment with various reagents, the microsome pellet derived from 1 gm. wet weight liver pulp was resuspended by homogenization as described and incubated in a total volume of 10 ml. under the conditions indicated in the tables. At the end of the incubation period the suspension was centrifuged for 2 hours instead of 1 hour at 105,000 *g*. The centrifugation time was extended in order to permit the sedimentation of smaller particles possibly derived from the microsomes as a result of the various treatments. In the centrifugal field mentioned, particles of 25 mμ diameter and 1.3 density would sediment within 2 hours according to Pickels' formula. After centrifugation the supernatant fluid and the sediment were separated by decanting. The sediment was resuspended in 10 ml. 0.88 M sucrose and the supernatant was brought up to the same volume. Both preparations were then divided into two 5 ml. aliquots on which chemical determinations were carried out in duplicate. Thus in each experiment four determinations were obtained on each of the chemical and biochemical constituents tested.

Methods.—To aliquots of pellet suspension and supernatant fluid, cold trichloroacetic acid (TCA) was added to a final concentration of 5 per cent. The TCA-precipitated material was then washed twice with cold, 5 per cent TCA. From this material, phospholipide, RNA, and the RNA-free and fat-free protein were obtained by the method of Schneider (35). The Mejbaum orcinol method (36) was used to estimate RNA quantitatively, using a standard sample of yeast-RNA (from Dr. Avery's laboratory) containing 0.090 mg. P per mg. In most experiments, data for RNA-phosphorus were also obtained by digesting the extracted RNA with 10 N H_2SO_4, and determining the phosphate of the digest by the Fiske-Subbarow method (37). The ratio of RNA phosphorus to RNA was lower than in the yeast RNA used as a standard, and varied in nearly all the experiments from 0.070 to 0.090. Because of this variation, and because the orcinol reaction is more sensitive than the Fiske-Subbarow reaction, only the figures for RNA are given in the tables. Protein nitrogen was determined by nesslerization (38), while phospholipide-phosphorus (PLP-P) was determined by digesting the alcohol-ether extract of the sample and determining the phosphate of the digest by the Fiske-Subbarow method (37). In the early experiments, the protein digest was also tested for the presence of phosphate, but since the extracted protein contained very little phosphate (less than 3 per cent of the combined RNA- and PLP-phosphorus), the values are not reported. This very low amount of residual phosphorus found in the protein may be either phosphoprotein-P, or RNA-P and PLP-P which escaped extraction.

The hemochromogen (30) was extracted by alcohol and alcohol-ether and the absorption shown by the extract at 400 mμ (absorption peak in alcohol) was used as a measure of the amount of pigment present.

Diphosphopyridine nucleotide–cytochrome *c* reductase activity was measured by the method of Hogeboom (31) at three different dilutions for each preparation. Only the linear parts of the time and concentration curves were used for determining the amount of enzymatic activity present in each preparation.

Materials.—The rats came from the Holzmann Rat Co., Madison, Wisconsin.

Diphosphopyridine nucleotide (DPNH) (75 per cent pure) and cytochrome *c* were obtained from the Sigma Chemical Co., St. Louis, the ribonuclease (RNA-ase) from the Nutritional Biochemical, Inc., Cleveland, and the deoxycholic acid from the Wilson Laboratories, Chicago. Immediately before use, the deoxycholic acid was dissolved in a NaOH solution and the ensuing solution of Na-deoxycholate was carefully back-titrated to pH 7.5–7.7.

Electron Microscopy

1. Liver in Situ.—The small fragments of liver tissue which served as intracellular control for these experiments were fixed for 2 hours at 0°C. in 1 per cent osmium tetroxide (OsO_4) buffered at pH 7.3–7.5 with 0.028 M Na acetate–0.028 M Na veronal (39). They were rapidly

dehydrated in graded ethanols (40) and embedded in *n*-butyl methacrylate at 47°C. (41). Sections, cut at 20 to 50 mμ with the Porter-Blum microtome (42), were examined in an RCA (model EMU-2b) microscope provided with a 250 μ aperture in the condenser and with a 25 μ aperture in the objective lens. Electron micrographs were taken at original magnifications ranging from 6,000 to 14,000 and further enlarged photographically as desired.

2. Liver Homogenates.—Small aliquots (2 to 3 ml.) of liver homogenates were fixed by mixing with an equal volume of 2 per cent OsO_4 in 0.88 M sucrose. After 1 to 2 hours fixation at 0°C., the homogenate was centrifuged down in a tightly packed pellet which was subsequently cut into small fragments. The latter were dehydrated and embedded according to the technique used for tissue blocks. The microtomy and the electron microscopy were carried through as for section 1.

3. Microsomes.—Three different procedures were tried for the electron microscopy of microsomes. The third procedure (section 3 *c* following) was found the most satisfactory and was used for most of the experiments reported.

(*a*) An aliquot of microsome suspension was fixed by mixing it with an equal amount of 2 per cent of OsO_4 in 0.88 M sucrose. After 2 to 20 hours fixation at 0°C., the fixed microsomes were sedimented by centrifugation (30 minutes × 105,000 *g*) and then resuspended in distilled water. Droplets of suitably diluted suspensions of fixed microsomes were put on film-coated screens, allowed to dry in the air, and examined directly in the electron microscope. This simple technique has already been used for a number of studies on cell fractions (see Discussion, p. 191) but its usefulness is limited by artifacts introduced by the air-drying of the preparations which can yield information only about the general shape and size of the particles present in the suspensions examined. For observation of structural details and for measurements, the following methods proved more suitable.

(*b*) The microsomes were fixed as above and then washed, dehydrated by passing through graded ethanols, and impregnated with *n*-butyl methacrylate. For this purpose the microsome suspension was centrifuged at each step prescribed by the technique, the supernatant decanted, and the sedimented microsomes resuspended in the fluid required by the next step of the procedure. Finally a thick suspension of microsomes in *n*-butyl methacrylate, with catalyst added, was allowed to polymerize at 47°C. The microtomy and microscopy were carried through as for section 1.

This technique has the advantage of processing the microsomes in essentially the same way liver cells are processed in tissue blocks. In addition, the microsomes are examined in sections and thus can be directly compared to intracellular structures found in hepatic parenchymatous cells, which are also observed in sections. The repeated centrifugations required by the technique result, however, in such severe losses in pellet material that it may be doubted whether the final preparation is still representative of the original microsome pellet. In addition, the technique is tedious and time consuming. All these disadvantages are obviated by the following procedure:—

(*c*) After the microsomes were sedimented, the supernatant was discarded, the plastic tube cut open, and the microsome pellet removed *in toto* with a fine spatula and placed in a drop of fixative solution. When this operation was done immediately after centrifugation, the pellet was so cohesive that it could be removed as a whole and did not disperse in the fixative solution or in the other subsequent media required by the procedure. After removal, the pellet was cut with a razor blade into small fragments which were fixed, dehydrated, and embedded as small blocks of tissue. For checking the homogeneity of the pellets, the blocks were cut in a prismatic form with the smallest side representing a section through the entire depth of the pellet; the prisms were properly orientated in the embedding matrix and sections were cut parallel to the smallest surface. Osmium tetroxide (1 and 2 per cent) in a variety of vehicles (*e.g.*, distilled water; 0.028 M Na acetate-0.028 M Na veronal, pH 7.3–7.5; 0.05 M phosphate buffer, pH

7.5; 0.15 M NaCl; 0.15 M KCl; 0.25 M sucrose; 0.88 M sucrose) was used for the fixation of the microsome pellets. The fixation was carried out at 0°C. and lasted from 2 to 20 hours.

4. Experimentally treated microsomes were processed according to the procedure under section 3 *c*. No difficulty was encountered in fixing pellets of washed, versene-treated and ribonuclease-treated microsomes. The pellets of microsomes treated with high concentrations of deoxycholate (0.5 per cent) disintegrated sometimes during fixation in OsO_4 solutions. Such pellets could be appropriately fixed with either formaldehyde (10 per cent), or ethanol, or saturated solution of $HgCl_2$, or 3 per cent potassium dichromate.

5. Postmicrosomal fractions were processed as the microsome fraction under section 3 *c*.

6. Final Supernatant.—A small aliquot was mixed with an equal volume of 2 per cent OsO_4 in 0.88 M sucrose. After 20 hours fixation at 0°C., the mixture was centrifuged for 60 minutes at 105,000 *g*. A brown, translucent pellet was obtained which was thereafter dehydrated, embedded, and sectioned in the same way as a microsome pellet.

RESULTS

Morphology of the Cytoplasm in Parenchymatous Liver Cells

The cytoplasm of the parenchymatous cells of the liver contains numerous mitochondria, a well developed endoplasmic reticulum, small dense particles, lipide inclusions, and other unidentified structures all embedded in a continuous, amorphous or finely granular matrix (Fig. 1).

In sections, the endoplasmic reticulum is represented by numerous profiles of circular, oval, elongated, or irregular shape. Their smaller diameter measures 50 to 150 mμ. They are bounded by a thin (~5 mμ) membrane and have an apparently homogeneous content which varies in density from one element to another. As in many other cell types (25 to 27), these profiles can be differentiated into a smooth surfaced and a rough surfaced variety. The profiles of the latter variety are characterized by close association with small (10 to 15 mμ in diameter), dense particles which appear to be attached to the outside surface of their limiting membrane. The membrane of the smooth surfaced profiles is free of attached particles.

The smooth surfaced profiles are of small size (40 to 100 mμ in diameter) and of circular, oval, or irregular shape. They occur in irregular agglomerations usually located towards the periphery of the cell. Within these agglomerations, the profiles are crowded, randomly disposed, and show numerous branchings and interconnections (Fig. 3). All these features indicate that in three dimensions the smooth surfaced profiles correspond to vesicles and short, contorted tubules linked together in a tightly meshed, randomly disposed reticulum.

The rough surfaced profiles are more numerous and among them the elongated forms are predominant. The length of the profiles is highly variable (from 50 mμ to 5 μ), but their small width or diameter (~50 mμ) is fairly constant. They frequently occur in arrays within which they are disposed parallel to one another at more or less regular intervals (Fig. 4). The examination of sections of various incidence as well as the analysis of serial sections indicate that some of these elongated profiles are longitudinal sections through tubules, but that

most of them represent perpendicular sections through cisternae; *i.e.*, flat vesicles of considerable width and length and of small and fairly uniform depth (25).

In parenchymatous liver cells, the two types of profiles are frequently found in clearly recognizable continuity (Fig. 2), a finding which shows that the two varieties of profiles represent local differentiations within a common system. The frequent parallelism of the rough surfaced profiles indicates that a considerable degree of preferred orientation prevails within the parts of the endoplasmic reticulum so differentiated.

In sections, the masses of smooth surfaced profiles and the arrays of rough surfaced ones appear separated by relatively large spaces, occupied by a finely granular material of low density. This material, presumably glycogen, diminishes noticeably in amount during fasting.

Most of the small dense particles present in the cytoplasm of parenchymatous liver cells occur in close association with the membrane of the endoplasmic reticulum and confer to parts of the latter the rough surfaced appearance mentioned. Sections cutting very obliquely through larger cisternal elements reveal that at the surface of their limiting membrane the particles are arranged in patterns among which circles and double rows appear to predominate. Relatively few particles occur freely scattered in the cytoplasm in between and around the rough surfaced elements of the endoplasmic reticulum.

In the vicinity of bile capillaries, the cytoplasm contains bundles of closely approximated, elongated profiles accompanied by swarms of small, circular profiles. In their detailed morphology, these profiles are similar to those of the smooth surfaced variety; they differ in being more tightly packed and usually exhibit a high degree of preferred orientation. Similar structures have been noted in the centrosphere region of many other cell types (25, 27), and were found to attain a considerable development in perikarya in which they have been described as the "agranular reticulum" (43). In epididymal epithelium (44), nephron epithelium (45), and in pancreatic acinary cells (46) structures of comparable appearance have been described as the unique or the main component of the Golgi apparatus.

Finally, throughout the cytoplasm but especially in the vicinity of the bile capillaries there occur spheroidal bodies of high density and compact appearance (Fig. 5). Some of them are structureless at the present level of resolution and, being identical in appearance with lipide droplets in other cells, are identified as lipide inclusions. Other dense bodies, however, have a limiting membrane and a fine granular texture of variable grain (Fig. 6). Their chemical nature and their role in the economy of the cell are completely unknown.

The cytoplasmic matrix, in which all the structures described are embedded, is not homogeneous but differs in character from one region of the cell to another. For instance, in the central regions, it consists mainly, as already men-

tioned, of a fine granular material of low density, whereas toward the periphery of the cell it is more dense and appears either finely fibrillar or amorphous at the present level of resolution.

The preceding description of the cytoplasm of parenchymatous liver cell is in good agreement with that recently published by Fawcett (47). The only difference, in relation to the endoplasmic reticulum, concerns the existence of a smooth surfaced part of the network in normal rats. Fawcett found that such a part appeared only in animals refed after prolonged fasting.

Morphology of Liver Homogenates

Almost all the structures described in the cytoplasm of parenchymatous liver cells *in situ* can be recognized in sections of pellets prepared from liver homogenates as indicated under Methods. These pellets exhibit a distinct layering: cell fragments and nuclei form the bottom layer, whereas the other cell components are found in appropriate succession in the upper layers. There is, however, no sharp separation between the layers of a pellet and, moreover, each of them consists of a mixed population of cell components with one component in majority.

Some of the cytoplasmic components are readily identified because their general shape and characteristic structural details are not altered by the homogenization of the tissue. Such is the case for mitochondria which still possess their two membranes and their cristae. Other components are altered during homogenization but the alterations are so limited and of such nature that the components concerned can still be satisfactorily identified. The sections of homogenate pellets contain, for example, "hollow" profiles comparable in size, shape, and number to the profiles of the endoplasmic reticulum in intact cells. Among them the two varieties described *in situ* can be easily recognized because many of these profiles still bear, attached to the outside surface of their limiting membrane, small, dense particles of 10 to 15 mμ in diameter (Fig. 7). This characteristic structural detail is taken to indicate that the profiles concerned derive from the rough surfaced parts of the endoplasmic reticulum. As inside the cytoplasm of intact cells, the rough surfaced profiles found in homogenate pellets are mostly of elongated shape and of small (*i.e.*, 50 to 70 mμ), even diameter. In three dimensions also, they correspond to cisternae and tubules. At variance with the situation encountered *in situ*, however, the profiles appear to be generally shorter, their preferred orientation is completely lost and, in the pellet, they occur indiscriminately mixed with other cytoplasmic components. The smooth surfaced profiles found in sections of homogenate pellets vary in size from 50 to 300 mμ, are of predominately circular and oval shape and appear to correspond in three dimensions to vesicles and short tubules. As *in situ*, profiles partly covered with, and partly free of, particles are occasionally encountered. The smooth surfaced vesicles of the homogenate derive, in all probability,

from the smooth surfaced parts of the endoplasmic reticulum. But, in the absence of a characteristic "label," their correlation with intracellular structures is less certain than in the case of the particle-labelled, rough surfaced profiles. It is conceivable that some of these smooth surfaced elements may represent "Golgi vesicles" or "Golgi granules" (44) dispersed during homogenization.

It can be concluded from these comparative observations that the homogenization of parenchymatous liver cells alters neither the fine structure, nor the size and general shape of the elements of the endoplasmic reticula. The operation mentioned appears to cause, however, an extensive fragmentation of the networks into independent vesicles, tubules, and cisternae, which subsequently can be centrifuged down, together with other cell components, into the homogenate pellets.

With few exceptions, the "hollow" profiles found in the homogenate are limited by a continuous membrane which does not show signs of breakage by tearing or fracture. In addition, the profiles have an apparently homogeneous content, which varies in density to a certain extent from one profile to another and which, although usually light, is frequently denser than the embedding plastic. These features may provide some indications about the mechanism involved in the fragmentation of the reticula.

In addition to mitochondria and fragments of the endoplasmic reticula, lipide inclusions and dense bodies of granular texture can be recognized in homogenate pellets. The fine fibrillar and granular elements of the cytoplasmic matrix have not been satisfactorily identified in the same preparations.

The Microsomes

A. Morphology.—The morphology of the microsome fraction was studied to advantage on sections of pellets fixed and embedded *in toto* as indicated under Methods (section 3 *c*).

In such sections, the predominant structural element is represented by membrane-bound profiles of approximately the same size and shape as the profiles found in homogenate pellets and considered to be derived, by extensive fragmentation, from the endoplasmic reticula of parenchymatous liver cells (Fig. 8). Among these profiles those of the rough surfaced variety retain their elongated form and small, uniform diameter and appear to correspond in three dimensions either to small cisternae or to tubules. These profiles bear attached to their membrane numerous, dense particles of 10 to 15 mμ in diameter (Figs. 8 and 10), and favorably oriented sections show that the particles are still disposed in patterns, mainly in double rows and circles, at the surface of the membrane limiting the profiles. The particles appear to be, however, slightly less numerous than around the corresponding profiles found in homogenates and intact cells, and consequently it must be assumed that a limited number of particles are detached during the separation procedure. Apparently "free"

particles, isolated or in clusters, are occasionally encountered in the microsome pellets, but it is difficult to ascertain whether they are actually free or appear so because of the particular orientation of the section.

Smooth surfaced profiles are less frequently encountered than rough surfaced ones in microsome pellets. They vary in size from 50 to 500 mμ and some of them, in comparison with corresponding structures in homogenate pellets and intact cells, appear to have suffered a certain amount of swelling. Profiles of mixed appearance, partly smooth and partly rough surfaced, are occasionally encountered among the ones already described (Figs. 8 and 10).

Finally, the microsome pellets contain a few small, dense bodies of granular texture and compact appearance of the type usually encountered in the vicinity of bile capillaries in intact cells (Fig. 9).

The microsome pellets appear to be relatively homogeneous, although a certain amount of layering can be detected. For instance, the larger fragments of the endoplasmic reticulum are found at the bottom of the pellet, whereas the smaller fragments predominate in the top layer which, in addition, appears to be richer in elements of the smooth surfaced variety.

The various structural elements found in pellet sections can be recognized in dried droplets of microsome suspensions (section 3 *a*); such preparations, however, do not give direct information about a number of structural details such as the "hollow" nature of most of the microsomal elements, the thickness of their limiting membrane, and the exact position of the small particles in relation to the membrane.

It is admittedly difficult to evaluate how much of the microsome fraction is accounted for by each of the structural elements described. By counting, it is found that in sections of pellets there are approximately 85 rough surfaced profiles for 13 smooth surfaced ones and 2 dense, compact bodies. Although such counts must be taken only as rough estimates, they indicate nonetheless that the dense compact bodies represent a minor constituent of the microsome fraction and that this latter consists mainly of fragments of the endoplasmic reticulum.

If the morphological analysis is pursued further, it appears that there are two major components in the microsome fraction; namely, (*a*) a membranous component represented by the membrane limiting the vesicles, tubules, and cisternae of the endoplasmic reticulum and (*b*) a particulate or granular component represented by the small, dense particles attached to the membrane of the rough surfaced elements of the reticulum. Such a distinction may appear arbitrary in the case of the liver, but it is justified by observations on many other cell types which indicate that the two components concerned, *i.e.*, the membrane of the endoplasmic reticulum and the particles, are fundamentally distinct structures.

The preceding description concerns microsomes isolated in 0.88 M sucrose and fixed in 1 or 2 per cent OsO_4 in the same concentration of sucrose. If the fixation is carried through at lower osmolar concentrations, the general appearance of the microsomes changes. After fixation in OsO_4 in 0.25 M sucrose ("isotonic"), the number of elongated, slender profiles is already smaller; after OsO_4 in 0.15 M NaCl, such elements are, as a rule, absent, having been replaced by large oval or circular profiles. Similar appearances are encountered in microsome pellets fixed in OsO_4 in 0.028 M Na acetate–0.028 M Na veronal (Fig. 11), and even larger vesicles are present in pellets fixed in OsO_4 in distilled water (Fig. 12). These large vesicular elements can be easily correlated with the tubular and cisternal elements found *in situ* and in microsome pellets fixed at 0.88 M sucrose because the vesicles still bear small, dense particles attached to their surface after all the alterations they suffered in size and general shape. Even when fixed at low osmolar concentrations, the microsome vesicles rarely show evidence of membrane discontinuity.

Structural elements of small size still remain in the final supernatant after the separation of the microsome fraction. The pellets obtained by centrifuging the final supernatant, after mixing it with fixative solutions, consist of a small number of "hollow" profiles of the smooth surfaced variety, and a small number of small, dense, apparently free particles, all embedded in a mass of amorphous, relatively dense material.

B. Chemistry.—Data concerning the chemical and biochemical components of the microsome fraction are given in Table I. The amounts of RNA and protein were similar to (6, 28) or smaller than (48, 49) those reported by other workers but the RNA/protein ratios were in good general agreement. The amount of phospholipide phosphorus was comparable to (50) or less than (33), that found by others. The latter situation applies also for the total and specific activity of DPNH-cytochrome *c* reductase (31). This enzyme, shown by Hogeboom (31) to be more concentrated in the microsomes than in the other cell fractions, was used in these experiments as a measure of microsomal enzymatic activity under various conditions.

The microsomes also contained a hemochromogen soluble in alcohol in which it showed a sharp absorption peak at 400 mμ and a smaller peak at 500 mμ. In deoxycholate extracts (0.5 per cent deoxycholate) the main peak of the pigment was shifted to 415 mμ and three much smaller peaks appeared at 350, 540, and 575 mμ respectively. This hemeprotein is probably identical with the one described by Strittmatter and Ball (29, 30) as a "cytochrome m," distinct from the other cytochromes of the cytoplasm.

In two experiments the various compounds mentioned were determined in the whole homogenates as well as in the microsome fractions derived therefrom. In these cases approximately 12 per cent of the total protein N, 33 per cent of the RNA, and 22 per cent of the phospholipide phosphorus of the homogenates were recovered in the microsome fractions. The microsomes were characterized

in addition by the highest RNA/protein ratio and the highest phospholipide/protein ratio of any of the cellular fractions examined (*cf.* references 7, 8).

Postmicrosomal Fractions

Because of the occurrence of a certain number of "free" dense particles in the cytoplasm, and because of the possible detachment of some particles from the membrane of the endoplasmic reticulum during homogenization, attempts were made to frac-

TABLE I

Biochemical Composition of Microsomal and Postmicrosomal Fractions of Rat Liver

First Section.—The figure for DPNH-cytochrome *c* reductase activity is an average of 5 experiments with standard deviation; all other figures are averages of 10 experiments with standard deviations.

Second Section.—Comparison between liver homogenates and microsome fractions derived therefrom. The figures are averages of two experiments.

Third Section.—Comparison between the usual microsomal fraction (M), two postmicrosomal fractions and the final supernatant. The figures represent a single experiment. M = sediment obtained after centrifuging a mitochondrial supernatant for 1 hour at 105,000 *g*; PM_1 = sediment obtained after centrifuging the supernatant of M for 3 hours at 105,000 *g*; PM_2 = sediment obtained after centrifuging the supernatant of PM_1 for 15 hours at 105,000 *g*; FS = supernatant of PM_2.

Fraction	Mg. protein N/gm.*	Mg. RNA/gm.*	μg. PLP-P/gm.*	$\frac{\text{Mg. RNA}}{\text{Mg. protein N}}$	$\frac{\mu\text{g. PLP-P}}{\text{Mg. protein N}}$	DPNH-cytochrome *c* reductase	
						Total act.‡/gm.*	Act./mg. N
Microsomes..	3.09 (±0.26)	3.46 (±0.31)	487 (±53)	1.12	157	1140 (±180)	369
Homogenate..	24.4	9.25§	1885	0.38§	77	—	—
Microsomes..	3.22	2.92	488	0.91	152	—	—
M	3.09	3.04	410	0.99	133	1130	366
PM_1	0.97	0.62	34	0.64	35	395	511
PM_2	0.92	0.36	11	0.39	12	—	—
FS	5.41	0.49	15	0.09	3	—	—

* Per gram wet weight liver pulp.

‡ μM cytochrome *c* reduced in 60 minutes.

§ These figures are high, since DNA also gives some color in the orcinol reaction.

tionate further the microsomal supernatant in the hope of obtaining a concentrate or a fraction of small particles. To this intent the supernatant was centrifuged for 3 hours at 105,000 *g* under the conditions described. The red, translucent pellet thus obtained was analyzed morphologically and chemically. The supernatant of this first postmicrosomal fraction was recentrifuged for 15 hours at 105,000 *g*. The pellet obtained was still red and translucent but it appeared less tightly packed than the preceding one and was covered by a fluffy layer of partly sedimented material. The pellet of this second postmicrosomal fraction was also analyzed morphologically and chemically. In addition, chemical determinations were carried out on the final supernatant.

Morphology.—In sections of fixed and embedded pellets, the first postmicrosomal fraction appeared composed of circular and oval profiles, smaller in size than those found in the microsome fraction (Figs. 13 and 14). The limiting membrane of these profiles appeared generally free of attached particles and few dense particles were encountered freely scattered in the pellet. The second postmicrosomal fraction consisted mainly of an amorphous, relatively dense matrix within which were found occasional profiles of small vesicular elements and a few small dense particles (Fig. 15).

Chemistry.—Chemical data shown in Table I indicate that the postmicrosomal fractions contain much less RNA and PLP-P than the microsomes. As the amount of protein does not decrease in the same proportion, the RNA/protein ratio and PLP-P/protein ratio drop precipitously in the postmicrosomal fractions. The first of them still exhibits an appreciable amount of DPNH–cytochrome *c* reductase activity and, moreover, its specific activity appears to be higher than that of the microsome fraction. The final supernatant still contains approximately 40 per cent of the cell proteins, and nearly 10 per cent of its RNA; its phospholipide content is negligible.

This correlated chemical and morphological analysis of the postmicrosomal fractions indicates that nothing is to be gained towards the separation of small particles by using conventional centrifugation techniques. It seems that such particles as are still present in the microsomal supernatant are centrifuged down together with small vesicles and with some proteins of high molecular weight usually considered as part of the final supernatant.

Various Treatments of the Microsome Fraction

1. Washing and "Aging".—The effect of washing the microsomes with 0.88 M sucrose, and of "aging" microsomal suspensions at various temperatures, *e.g.* 0°, 30°, and 37°C., for periods of 30 and 60 minutes, was investigated in order to find out whether such treatments result in the preferential removal of any structural or chemical constituent of the microsome fraction.

The pellets of washed or "aged" microsomes retained the translucence and the red color of the usual microsome pellets, and appeared to be only more tightly packed. Electron micrographs of sections of fixed and embedded pellets showed that the treatments mentioned induced a further fragmentation of the microsomes which retained, however, most of their structural characteristics. Washing and especially aging caused a further reduction in the number of recognizable, attached particles. The latter were apparently replaced by irregular thickenings of, and deposits on, the limiting membrane of the vesicles.

The corresponding chemical information is presented in Table II, which gives for each component the amount lost from the pellet as a percentage of the amount contained in an untreated control pellet. The amount lost to the supernatant as a result of the treatment was also determined and recoveries close to 100 per cent were obtained for all components investigated with the exception of the enzyme activity. In the case of the latter, total recovery ranged from 70 to 90 per cent; the net loss might be due to the inactivation of the enzyme during "aging" (31). It can be seen that, in contrast to the many reported examples of mitochondrial instability, the microsome fraction was remarkably

stable even when incubated at 37°C. for 60 minutes. In general the losses in RNA and PLP-P did not exceed 10 to 15 per cent and the losses in protein, although more variable, remained usually under 20 per cent. Only in the

TABLE II

Biochemical Stability of Liver Microsomes

The microsomes were obtained from rat liver homogenized in 0.88 M sucrose (centrifugation: 60 minutes at 105,000 *g*). They were resuspended either in H_2O, or 0.88 M sucrose, or 0.88 M sucrose with the additives given, and incubated for the time and at the temperatures indicated. The suspensions were recentrifuged thereafter for 120 minutes at 105,000 *g*, and the pellets analyzed chemically. The losses incurred during treatment are given as per cent of the amounts found in the original microsome fraction.

Conditions of treatment	Loss from pellet				
	Protein N	RNA	Phospho-lipide-P	Hemochro-mogen	DPNH-cytochrome *c* reductase activity*
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
30 min., 0°, H_2O	16	6	0	3	—
30 min., 0°, 0.88 M sucrose	13	12	10	12	—
	19	8	16	14	15
60 min., 0°, 0.88 M sucrose	27	15	17	15	—
	13	3	11	7	15
30 min., 30°, 0.88 M sucrose	16	13	11	21	—
30 min., 37°, 0.88 M sucrose	28	13	10	20	16
60 min., 37°, 0.88 M sucrose	18	10	13	17	20
60 min., 30°, 0.88 M sucrose 0.05 M tris,‡ pH 7.3	39	45	24	72	—
0 min., 0°, 2 per cent versene (pH 7.5) in 0.88 M sucrose	38	57	15	32	41
60 min., 0°, 2 per cent versene (pH 7.5) in 0.88 M sucrose	47	67	19	69	48

* These figures are approximate, since total recovery of activity in pellet and supernatant could not be obtained after treatment.

‡ Tris(hydroxymethyl)aminoethane-maleate buffer.

presence of the "tris" buffer was a sizeable proportion of the various components lost, but even in this case none of the components was lost preferentially from the pellet. The results suggest that in the presence of the "tris" buffer a large number of microsomes begin to disintegrate as a whole.

2. Versene Treatment.—Isolated microsomes were resuspended in 0.88 M

sucrose containing 2 per cent versene (pH 7.5) and resedimented either immediately or after 1 hour incubation at 0°. The pellet obtained was brown and opaque while the supernatant was red and clear. In sections the pellet appeared to be formed of numerous vesicles of various sizes bearing a small number of particles attached to their membrane and associated with some amorphous material (Fig. 16). Chemical data (Table II) indicate considerable loss in RNA (60 to 70 per cent) and hemochromogen with a smaller loss in protein.

3. Deoxycholate Treatment.—

Deoxycholate is a surface-active agent which has been used extensively in microbiology for disrupting bacterial cells and isolating, among others, "transforming agents"; *i.e.* nucleic acid complexes of macromolecular dimensions (51). In biochemistry it has been used for the solubilization of mitochondrial cytochromes (52) as well as for the solubilization of microsomal hemochromogen (29, 30) and glucose-6-phosphatase (53).

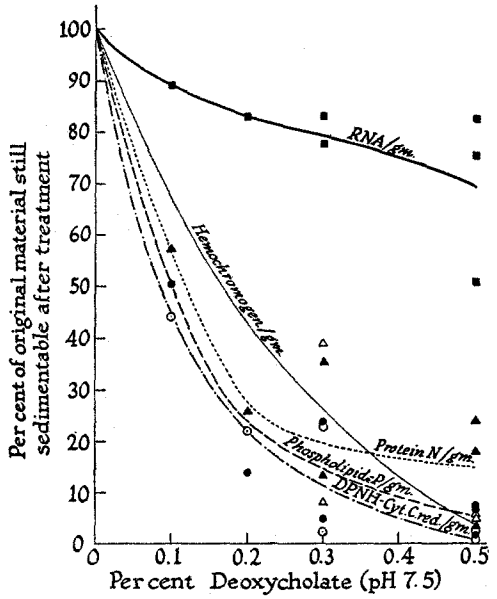
Suspensions of microsomes in 0.88 M sucrose became clear upon the addition of 5 per cent Na deoxycholate adjusted to pH 7.5. The clarification was rapid for final concentrations of 0.1 or 0.2 per cent and instantaneous for concentrations of 0.5 per cent. After recentrifugation for 2 hours at 105,000 *g* the clarified suspensions yielded small, slightly opaque, colorless or yellowish pellets. The clear supernatant was red colored. Usually the pellets of deoxycholate-treated microsomes could be fixed, as the other fractions, in OsO_4 solutions but sometimes pellets obtained after treatment with relatively high deoxycholate concentrations (*i.e.* 0.5 per cent) disintegrated either during fixation or during dehydration. Such pellets could be satisfactorily fixed with formaldehyde, potassium dichromate, or ethanol.

In sections, the pellets were found to consist mainly of small, dense particles of rounded or polygonal shape, measuring 10 to 15 mμ in diameter and therefore morphologically identical with the particles previously found attached to the outer surface of the membrane limiting the microsomal vesicles (Fig. 18). Profiles of membrane-bound elements were only occasionally encountered and large fields of the pellets contained none. Such vesicles as present were usually of small size and rarely have attached particles. The general morphology of the particles did not appear to be noticeably influenced by the fixative employed. They retained their shape, size, and relatively high density when fixed in formaldehyde or potassium dichromate.

The appearance of the pellets varied to a noticeable extent with the concentration of deoxycholate used in the treatment of microsome suspensions. The pellets obtained from suspensions treated with 0.1 per cent deoxycholate still contained a relatively large number of vesicles. The membrane-limited elements decreased rapidly in number with increasing concentrations of reagent and were only occasionally encountered after 0.3 to 0.5 per cent deoxycholate. In the latter case, however, a new structural component was frequently present

in the pellets; it appeared as a filamentous structure of ~4 to 5 mμ diameter usually disposed in skeins.

These findings are taken to indicate that the deoxycholate disrupts the vesicles and solubilizes the material of their limiting membrane, leaving behind the small dense particles in a still recognizable form. The origin of the fine fibril-



TEXT-FIG. 1. Effect of deoxycholate on the biochemical composition of liver microsomes. The microsomes were obtained from rat liver homogenized in 0.88 M sucrose (centrifugation: 60 minutes at 105,000 *g*). They were resuspended in 0.88 M sucrose containing the amounts of Na deoxycholate (pH = 7.5) indicated. The treated suspensions were recentrifuged immediately for 120 minutes at 105,000 *g* and the pellets thus obtained were analyzed chemically. The results, given per gram tissue equivalent, indicate what per cent of the original material is still sedimentable after deoxycholate treatment.

■———■, RNA; ▲- - - - - -▲, protein N; ●— — —●, phospholipide P; △———△, hemochromogen; O— - — -O, DPNH-cytochrome *c* reductase activity.

lar material found in the pellets obtained after treatment with 0.5 per cent deoxycholate is more difficult to ascertain. It may derive from the small dense particles as a result of their disintegration at higher deoxycholate concentrations.

The results of the deoxycholate treatment were also followed chemically by determining the distribution of the various microsomal components in the pellets and the respective supernatant fluids. The graph in Text-fig. 1 indicates that most of the microsomal RNA could be recovered in the pellets whereas most of the other components, *i.e.* the protein, phospholipide, hemochromogen, and enzyme, were lost to the medium. The RNA recovery amounted to 80 to 90

per cent and seemed little influenced by the deoxycholate concentration, whereas the loss of the other components increased rapidly with the concentration and reached 75 to 95 per cent at 0.3 and 0.5 per cent deoxycholate. As in the previous experiments the materials lost from the pellet could be satisfactorily accounted for in the supernatant with the exception of the enzyme which was apparently partially inactivated by the deoxycholate. In the case of the nucleoprotein, the relatively small loss could be accounted for still as nucleoprotein and not as any of its degraded products. The RNA/protein ratios of the pellets are shown in Table III. It can be seen that in general they increase with deoxycholate concentration but that a large spread is obtained at 0.3 and 0.5 per cent deoxycholate.

TABLE III

Effect of Deoxycholate Treatment on the RNA/Protein N Ratio of Liver Microsomes

The microsomes were isolated from rat liver homogenized in 0.88 M sucrose (centrifugation: 60 minutes × 105,000 *g*). They were resuspended in 0.88 M sucrose containing the amounts of deoxycholate (pH = 7.5 to 7.7) indicated. These suspensions were recentrifuged for 120 minutes at 105,000 *g* and the pellets analyzed for RNA and protein N.

Concentration of deoxycholate	0	0.1 per cent	0.2 per cent	0.3 per cent	0.5 per cent
$\frac{\text{mg. RNA}}{\text{mg. protein N}}$	0.99	1.52	3.13	—	—
	1.40	—	—	7.97	—
	1.37	—	—	3.22	—
	1.04	—	—	—	8.95
	1.02	—	—	—	4.65
	1.05	—	—	—	3.35

The correlation of morphological and chemical data is taken to indicate that the RNA is located in the small particles which appear to be made up mainly of nucleoprotein. The other components, *e.g.* phospholipide, hemochromogen, enzyme, and most of the protein, are solubilized by the deoxycholate at the same time that the membranous component of the microsomal fraction disintegrates. It is assumed that these components are part of the membrane itself or part of the content of the microsomal vesicles.

4. Ribonuclease Treatment.—Finally, microsome suspensions were incubated in various concentrations of crystalline ribonuclease at the temperatures and for the periods shown in Table IV. The incubation at higher temperatures caused a heavy agglutination of the microsomes, visible to the naked eye.

The centrifugation of incubated suspensions yielded pellets only slightly smaller than the original microsome pellets, but opaque and of brown color, the clear supernatant being colorless, or slightly pink.

In the electron microscope, all pellets obtained by recentrifuging RNA-ase–treated microsomes were found to consist of swollen, tightly agglutinated

vesicles. After incubation at 37°C., the agglutinated vesicles, which occluded a certain amount of amorphous material among them, appeared free of attached particles (Fig. 17). The same appearance was encountered after incubation at 30°C., but the amount of amorphous material seemed larger. Finally after incubation at 4°C., attached particles were found present, on the outer

TABLE IV

Effect of Ribonuclease on the Biochemical Composition of Liver Microsomes

The microsomes were isolated from rat liver homogenized in 0.88 M sucrose (centrifugation: 60 minutes at 105,000 *g*). They were resuspended in 0.88 M sucrose containing the amounts of RNA-ase indicated. The suspensions were incubated for the time and at the temperatures given and centrifuged thereafter for 120 minutes at 105,000 *g*. The pellets thus obtained were analyzed chemically. The figures in the table indicate the effect of the treatment in terms of per cent deviation from controls. The latter were incubated under identical conditions but without RNA-ase.

Conditions	RNA-ase per ml.	Per cent change from control pellet				
		Protein N	RNA	Phospholipide-P	Hemochromogen	DPNH-cytochrome *c* reductase activity*
	mg.					
0.88 M sucrose; 0°, 30 min.	0.5	0	−27	+4	−1	—
	0.5	+10	−8	+14	+1	−20
0.88 M sucrose; 0°, 60 min.	0.5	+10	−19	+10	+6	−27
	0.5	+3	−32	+5	+6	−26
0.88 M sucrose; 30°, 30 min.	0.1	0	−37	+10	0	—
0.88 M sucrose; 0.05 M tris buffer pH 7.3 30°, 30 min.	0.01	−11	−44	+3	−10	—
	0.02	−6	−49	+1	−4	—
	0.05	+16	−63	+3	−4	—
0.88 M sucrose; 37°, 30 min.	0.5	−6	−83	+21	−29	−48
0.88 M sucrose; 37°, 60 min.	0.5	−17	−85	+8	−31	−26

* Figures are approximate, since total recovery of activity in pellet and in supernatant was less in the RNA-ase experiments than in the sucrose controls.

aspect of the vesicles, but a precise observation of these particles and a satisfactory evaluation of their number was prevented by the intensity of the agglutination. The controls incubated in sucrose alone showed a loss in attached particles that was small after incubation at 4°C. and appreciable after incubation at 30° and 37°C. In these cases some of the particles were apparently replaced by irregular thickenings of the membrane and by amorphous deposits thereon. The effects of ribonuclease upon the chemical composition of the microsomes is shown in Table IV. The figures indicate, in terms of per cent

deviation from the controls, the amount of change, *i.e.* loss or gain, recorded in the pellets of treated suspensions. The controls were microsome suspensions incubated at the same temperature and for the same time in 0.88 M sucrose alone. It can be seen that losses in RNA are small after incubation at 0°C.; reach approximately 40 per cent at 30°, and surpass 80 per cent at 37°C.

According to Schmidt *et al.* (54), RNA-ase acts upon the pyrimidine-phosphate ester bonds of the RNA molecule, the action of the enzyme being completed when 46 per cent of the RNA is hydrolyzed. With this in mind, it can be assumed that the effect noted in the experiments carried at 30°C. was due to RNA-ase action alone, whereas the further dissolution obtained at 37°C. was not directly related to the activity of the enzyme. It should be pointed out, however, that even at 30°C., after only 37 per cent of the RNA had been removed from the pellet, few recognizable granules were left on the microsomal membranes. The finding suggests that the intact RNA molecule is necessary for the maintenance of the granular structure of the nucleoprotein and that once its pyrimidine-phosphate ester bonds are disrupted, giving free pyrimidine monoester groups (54), the granular structure is lost.

Changes induced by RNA-ase treatment in the amounts of the other microsomal components were generally small and of rather difficult interpretation. The DPNH–cytochrome *c* reductase activity, for instance, decreases in the pellets after incubation in RNA-ase, the over-all loss being approximately 50 per cent higher than the loss incurred by controls. This decrease may be due either to a specific inhibition, or to some proteolytic activity in the RNA-ase preparations. It can be added, in this respect, that DPNH-cytochrome *c* reductase activity proved relatively resistant to the various agents tested. Neither aging, nor versene, deoxycholate, and RNA-ase treatment caused complete inactivation: they induced only a rather limited inhibition.

DISCUSSION

The morphological observations and the biochemical data presented in this paper bear on a number of problems relevant to the microsome fraction.

Correlation with Intracellular Structures.—The first of these problems concerns the nature of the microsomes and their relationship to intracellular structures. By systematically examining liver tissue, liver homogenates and microsome fractions derived therefrom, it was possible to demonstrate that the microsomes are fragments of the endoplasmic reticulum derived, to a large extent, from the rough surfaced parts of the network and, to a lesser extent, from its smooth surfaced regions. They represent therefore cytoplasmic structures known to preexist in the intact cell and consequently they are not artefacts introduced by tissue homogenization. Two factors have been particularly helpful in establishing the derivation mentioned. The first one was the use of the attached particles as a "label" in following the fate of the endoplasmic reticulum through the various steps of the homogenization and fractionation

procedure. The second factor was the introduction of the technique for processing fraction pellets *in toto* and examining them in sections. This technique allowed the comparison of structural elements present *in situ* and in the microsome pellets under the same geometrical conditions and at comparable levels of resolution. It proved both efficient and expeditious and it is accordingly recommended for future use as a routine technique for checking cell fractionation procedures with the electron microscope.

The finding that the microsomes derive from the endoplasmic reticulum confirms Claude's original hypothesis (3, 5, 9) that the microsomes are "preformed" cytoplasmic components. However, in the light of the present study, their "particulate" character, repeatedly stressed in the literature (5, 7), appears to be an artefact introduced by the homogenization of the tissue. *In situ*, the elements concerned usually occur not as independent bodies but as parts of a continuous, cell-wide system; *i.e.*, the endoplasmic reticulum. It has already been postulated (24, 26, 55) that the microsomes represent fragments of this reticulum and preliminary evidence on this point has been advanced by Porter (24). The present study confirms this postulation and establishes firmly the derivation of the microsomes from the endoplasmic reticulum by using more favorable conditions for observation, more reliable criteria for identification, and an appropriate material, *i.e.* the liver, which is currently the most common source of microsomes. The conclusions of the study do not support other hypotheses according to which microsomes are fragmented mitochondria (56), fibrillar components of the ground substance (57), or a group of particles arbitrarily isolated from a continuous spectrum of cytoplasmic granules (11).

Homogeneity.—Although the majority of the microsomes are fragments of the endoplasmic reticulum, it is evident that the microsome fraction is not homogeneous from a cytological standpoint. For instance, some of the smooth surfaced vesicles of the fraction may be derived from the cell membrane or from the agglomerations of membranous structures found in the vicinity of bile capillaries. Although similar structures have been identified as the Golgi apparatus in various cell types (44–46) including the parenchymatous cells of the liver (47), a few observations suggest that these vesicular agglomerations may represent a differentiated part of the endoplasmic reticulum (27, 43); if so, the vesicles derived therefrom may not be entirely different from the rest of the microsomes. The dense bodies of granular texture found in the microsome pellets represent, however, a different, apparently unrelated cytoplasmic component, and as such they render the microsome fraction cytologically heterogeneous. In this respect, the preceding observations support the views advanced by Hogeboom *et al.* (55, 58) and by Bernhard (59) but, at variance with their conclusions, it appears that the fraction is not so heterogeneous as to be of limited or questionable cytological significance. Actually the microsome fraction

can be considered as representative of the endoplasmic reticulum of hepatic cells[3] because elements derived from sources other than the reticulum make up only a small percentage of the microsome population.

The discrepancy between the view presented in this study and that taken by the authors mentioned (55, 58, 59) can be explained by differences in technique and interpretation. For instance, for the sake of "purity," the fluffy layer was not included (as frequently done) in our microsome fractions, and this omission can be expected to minimize contamination by mitochondria, mitochondrial fragments, and other particles of comparable size. In addition, no particular significance was attached in this study to the size of the microsomal elements (*cf.* 58, 59), because it was found that the disintegration of the endoplasmic reticulum results in fragments of highly variable sizes; *i.e.*, from a few micra to a few millimicra in length. Finally vesicular elements with and without attached particles were not considered as representing necessarily different cell organelles, because such elements were regularly found integrated in a common structure; *i.e.*, the endoplasmic reticulum in the intact, parenchymatous cells of the liver.

There are relatively few mitochondria, most of them extracted or fragmented, in the microsome pellets studied and little amorphous material of the type found in preparations obtained from the final supernatant. Consequently the microsome fraction appears to be relatively "pure" from the present cytochemical standpoint in the sense that its contamination by mitochondria and by supernatant material is small or negligible. The reverse is not true: both the mitochondrial fraction and the final supernatant are appreciably contaminated by microsomes.

Previous Morphological Studies on Microsomes.—The preceding description of the microsome fraction agrees with earlier findings by Slautterback (60) who, studying liver microsomes in dried droplets of microsome suspensions, described the fraction as a mixed population of "large, small, and dense, intermediate size particles." He recognized that the "large particles" were membrane-bound vesicles and that the "small particles" were dense bodies ~20 mμ in diameter. He found them usually in close association, but, because of unfavorable conditions for observation, was unable to ascertain their relative position and assumed that the small particles were contained in the large ones. Slautterback did not attempt to correlate the various microsomal forms with intracellular structures. In retrospect, it appears that his "large particles" correspond to the fragments of the endoplasmic reticulum; the "small particles" to the dense, particles attached thereto, and the "dense, intermediate size particles" to the peribiliary bodies described in this paper.

[3] The cell population of the liver being unhomogeneous, it follows that the endoplasmic reticula of many cell types (*e.g.*, parenchymatous liver cells, macrophages or Kupffer cells, epithelia of bile ducts, fibroblasts, etc.) are represented in the microsome fraction. The main source of microsomes remains, however, the endoplasmic reticulum of parenchymatous liver cells, because these latter are preponderant in number and mass.

There are in the cytological and cytochemical literature a number of less detailed morphological observations on microsomes isolated either from liver (33, 59, 61–63) or from other animal tissues (33, 59). According to these observations the microsomes are small vesicles (59, 61), granules (59), particles (62), or aggregates of particles (63) ranging in size from 10 to 250 mμ. Some observers found the microsome population rather homogeneous (62, 63), whereas others stressed its heterogeneity (59). It is assumed that discrepancies between these conclusions and the findings here reported are due primarily to differences in preparatory techniques for electron microscopy. All the observations mentioned were carried out on dried droplets of microsome suspensions under unfavorable conditions for satisfactory preservation and for observation of structural detail.

Fragmentation of the Endoplasmic Reticulum.—It is evident that the endoplasmic reticulum undergoes an extensive fragmentation during the homogenization of the tissue, but the factors involved in the process are still unknown.

It may be assumed that the fragmentation is due to mechanical factors at work during the grinding of the liver pulp, but it can be pointed out that the membrane of the microsomes shows no evidence of tearing or breakage. On the contrary, the microsomes generally appear to be limited by a continuous, apparently faultless membrane, and frequently possess a content higher in density than the embedding plastic. The osmometer-like behavior exhibited by the microsomes when fixed in media of low osmolar concentration is a further indication that their bounding membrane is continuous and semipermeable and that it imprisons a content of molecules of large size. If the fragmentation is due to mechanical factors, then it has to be admitted that the torn fragments "heal" easily—as myelin figures do when cut or torn apart (64). An alternative explanation of the fragmentation would be a "spontaneous," generalized pinching-off process of the endoplasmic reticulum that occurs before or during homogenization. The process may involve, as more immediate causes, anoxemia, incipient cytolysis, or changes brought about by damage to the cell membrane. In this respect it can be noted that a comparable fragmentation of the reticulum into independent vesicles is found at the periphery of tissue blocks in cells damaged or cut open during the trimming of the block. A similar fragmentation has been described by Porter (24) in cultured cells approaching cytolysis.

Integration of Morphological and Biochemical Information.—Although the microsomes represent a mixed population of cytoplasmic components, it can be assumed that the chemical composition of the fraction reflects to a large extent the chemistry of the major component, namely that of the vesicles, especially of the rough surfaced vesicles, derived from the endoplasmic reticulum. This view is supported by the scarcity of structural elements of different origin, and by the rather general character of most of the chemical components thus far investigated.

As a result of a more detailed morphological analysis, two major structural components are distinguished in the microsome fraction. One is a membranous component represented by the limiting membrane of the smooth and rough surfaced vesicles; it corresponds *in situ* to the limiting membrane of the endo-

plasmic reticulum. The other is a particulate component which, in this case, appears to be firmly attached to the outer aspect of the membrane limiting the majority of the vesicles. The relatively high density and the osmometer-like behavior of the microsomes suggest the existence of a third component represented by the apparently amorphous content of the microsomal vesicles. The association between the two major components, *i.e.* the particles and the membrane, is strong enough to resist tissue homogenization and fractionation procedures. Moreover, it is not markedly affected by "aging": "aged" microsome preparations show limited morphological alterations and only small, usually proportional losses of their chemical constituents. The particulate component is, however, noticeably affected by versene treatment, after which the microsome fraction consists mainly of vesicles with few attached particles. It may be assumed that the amorphous material found in such preparations around the vesicles is derived from particles damaged by versene. The findings suggest that metals, presumably divalent ions, are involved not only in the binding of the particles to the membrane but also in the binding of the material within the particles (*cf.* reference 65). Chemical analyses of versene-treated microsomes indicate losses of ~60 per cent in RNA and ~30 per cent in protein coupled with small or no losses in the original phospholipide content of the preparations. These results are in agreement with those reported by Tsuboi *et al.* (65) who found that after versene treatment more than 50 per cent of the microsomal RNA becomes unsedimentable.

Findings after versene treatment suggest that the RNA of the microsome fraction is located in the small, dense particles and the suggestion is amply confirmed by the results obtained with deoxycholate-treated microsomal suspensions. The rapid clarification of the microsome suspensions upon the addition of this reagent is already an indication of drastic change in size of the suspended elements. This indirect indication is borne out by direct findings on the pellets obtained by centrifuging deoxycholate-treated suspensions. Electron micrographs of such pellets show that the relatively large vesicular elements of the original microsomal preparations have disappeared and that the pellets consist mainly of small, dense particles 10 to 15 mμ in diameter, which in size, shape and density are similar to particles still attached to vesicles in untreated aliquots. The fact that particles of such small size can be sedimented under the conditions described (page 174) indicates that they either have a relatively high specific gravity, or are associated in small clumps. The chemical analysis of the pellets shows that these particles consist mainly of RNA and protein and carry with them ~80 to 90 per cent of the original microsomal RNA and ~20 per cent of the original protein. From the correlation of these morphological observations and chemical data it appears that the small dense particles are nucleoprotein particles and that the RNA of the microsome fraction is actually located in them. These results are in agreement with those recently reported by

Littlefield *et al.* (66) who were also able to recognize vesicles and small particles in the microsome fraction and to isolate the nucleoprotein particles after deoxycholate treatment. A point of disagreement between their findings and those here reported concerns the size of the particles which is greater in their case (~20 mμ). The discrepancy could be accounted for by differences in preparatory procedures prior to electron microscopy. Their preparations were unfixed and dried in the air, a procedure in which a certain increase in size by flattening is unavoidable.

The chemical components which are rendered unsedimentable by deoxycholate treatment can be ascribed to the membranous component which appears therefore to contain a large part of the protein and practically all the phospholipide, hemochromogen and DPNH–cytochrome *c* reductase of the microsome fraction. It should be noted however that some of these substances may actually be present in the content of the microsomal vesicles not in their limiting membranes. Thus far there are no data concerning the individual chemistry of these two components.

Although the deoxycholate affects primarily the membranous component, of the microsomal fraction, its action is far from specific. On one hand apparently intact vesicles are still encountered in small numbers in pellets of treated suspensions and, on the other hand, such pellets contain a fibrous material, in addition to small particles, especially after treatment with relatively high concentrations of deoxycholate. In such cases, the pellets are also characterized by a high RNA to protein ratio. It can be assumed that at higher concentrations the deoxycholate damages also, though to a lesser extent, the nucleoprotein particles by splitting off part or all of their protein. The fibrous material found in such preparations may represent either a nucleoprotein particularly rich in RNA or RNA alone.

The general scheme of localization proposed for the various chemical components of the microsomes is supported also by the results obtained with ribonuclease-treated microsomes. Under certain conditions, *i.e.* long incubation at 37°C., approximately 80 per cent of the microsomal RNA is "solubilized," a finding which confirms earlier observations by Novikoff *et al.* (67), and, at the same time, the particles are lost. Because of the severe agglutination induced by ribonuclease treatment, such pellets are, however, less favorable for detailed morphological observations.

Literature Bearing on the Nucleoprotein Particles.—The finding of a small particle richer in RNA than usual microsomes provides support for the earlier studies of Chantrenne (11) and Barnum and Huseby (6) who were able to separate postmicrosome or "ultramicrosome" fractions with a high RNA content. It may be assumed that in these cases, the authors mentioned were dealing with detached particles but it remains to be explained how the detachment was induced.

The particulate component of the microsome fraction is in all probability, identical

with the "macromolecules" described by Petermann *et al.* (12–14) as indicated by the high RNA content, the calculated size of the macromolecules, and by the fact that they were recently obtained also from deoxycholate-treated microsomes (68). Petermann and her collaborators succeeded in isolating the macromolecules and in differentiating them in a number of types by analytical centrifugation and electrophoresis of microsome suspensions. The procedures used are sensitive enough to detect the relatively few free particles usually found in microsome suspensions. It appears however that macromolecules were obtained in sizeable amounts, presumably after being detached from the vesicles at some step in the preparation procedures.

Terminology.—A final point to be discussed concerns the term microsome itself. This name, originally proposed by Claude for isolated "small granules," is now in general use and is frequently taken to designate a definite cell component existing in all types of cells (69). The present study shows that, apart from the heterogeneity of the microsome populations, the microsomes contain two structurally different components: one membranous and the other particulate in nature. These two components occur in close association in the case of the liver, but such an association is absent in many other cell types (26). Moreover the arrangement found in the liver does not seem to be of a basic, fundamental nature: it appears to represent a rather specialized development that the liver cells have in common with neurons (43) and glandular cells (26). It follows that microsomes separated from other tissues will not have necessarily the same structural components, and the same chemical composition as liver microsomes. For instance the high RNA content, considered as the characteristic biochemical feature of the microsomes, can be expected to vary with the amount of small particles attached to the endoplasmic reticulum. The RNA content of the microsomes may thus be very small for some tissues. It also follows that a survey of microsome structure and chemistry in a series of judiciously chosen cell types may help in elucidating our views on the problem. For the moment the most interesting development seems to be the morphological and chemical characterization of a new cytoplasmic component; *i.e.*, the small dense particles which apparently deserve the name of nucleoprotein particles. There are already interesting data suggestive of their activity in protein synthesis (66) and indicating their variability in growing cells, normal or neoplastic (12–14). Further studies of this component may contribute towards a better understanding of its association with the endoplasmic reticulum in cells involved in the production of proteins on a large scale.

SUMMARY

Rat liver, liver homogenates, and microsome fractions separated therefrom were examined systematically in the electron microscope in sections of OsO_4-fixed, methacrylate-embedded tissue and pellets.

It was found that most microsomes are morphologically identical with the rough surfaced elements of the endoplasmic reticula of hepatic cells. They appear as isolated, membrane-bound vesicles, tubules, and cisternae which contain an apparently homogeneous material of noticeable density, and bear small, dense particles (100 to 150 A) attached to their outer aspect. In solutions of various osmolar concentrations they behave like osmometers. The findings sug-

gest that they derive from the endoplasmic reticulum by a generalized pinching-off process rather than by mechanical fragmentation.

The microsome fractions contain in addition relatively few vesicles free of attached particles, probably derived from the smooth surfaced parts of the endoplasmic reticula. Dense, peribiliary bodies represent a minor component of the same fractions.

The microsomes derived from 1 gm. wet weight liver pulp contained (averages of 10 experiments) 3.09 mg. protein N, 3.46 mg. RNA (RNA/protein N = 1.12), and 487 μg. phospholipide P. They displayed DPNH–cytochrome *c* reductase activity and contained an alcohol-soluble hemochromogen.

The microsome preparations proved resistant to washing and "aging." Treatment with versene and incubation with ribonuclease (30 minutes at 37°C.) resulted in appreciable losses of RNA and in partial or total disappearance of attached particles.

Treatment with deoxycholate (0.3 to 0.5 per cent, pH = 7.5) induced a partial clarification of the microsome suspensions which, upon centrifugation, yielded a small pellet of conglomerated small, dense particles (100 to 150 A) with only occasionally interspersed vesicles. The pellet contained ~80 to 90 per cent of the RNA and ~20 per cent of the protein N of the original microsomes. The supernatant accounted satisfactorily for the materials lost during deoxycholate treatment.

The findings suggest that the microsomal RNA is associated with the small particles whereas most of the protein and nearly all of the phospholipide, hemochromogen, and DPNH–cytochrome *c* reductase activity are associated with the membrane or content of the microsomes.

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Addendum

While this article was in press, a correlated morphological and biochemical study of cell fractions derived from rat liver homogenates was published by Kuff, Hogeboom, and Dalton (*J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 33). The authors mentioned were not able to find small dense particles in the fraction containing most of the cytoplasmic RNA. It is hoped that the discrepancy between their findings and those reported in the present article will be explained by further work.

EXPLANATION OF PLATES

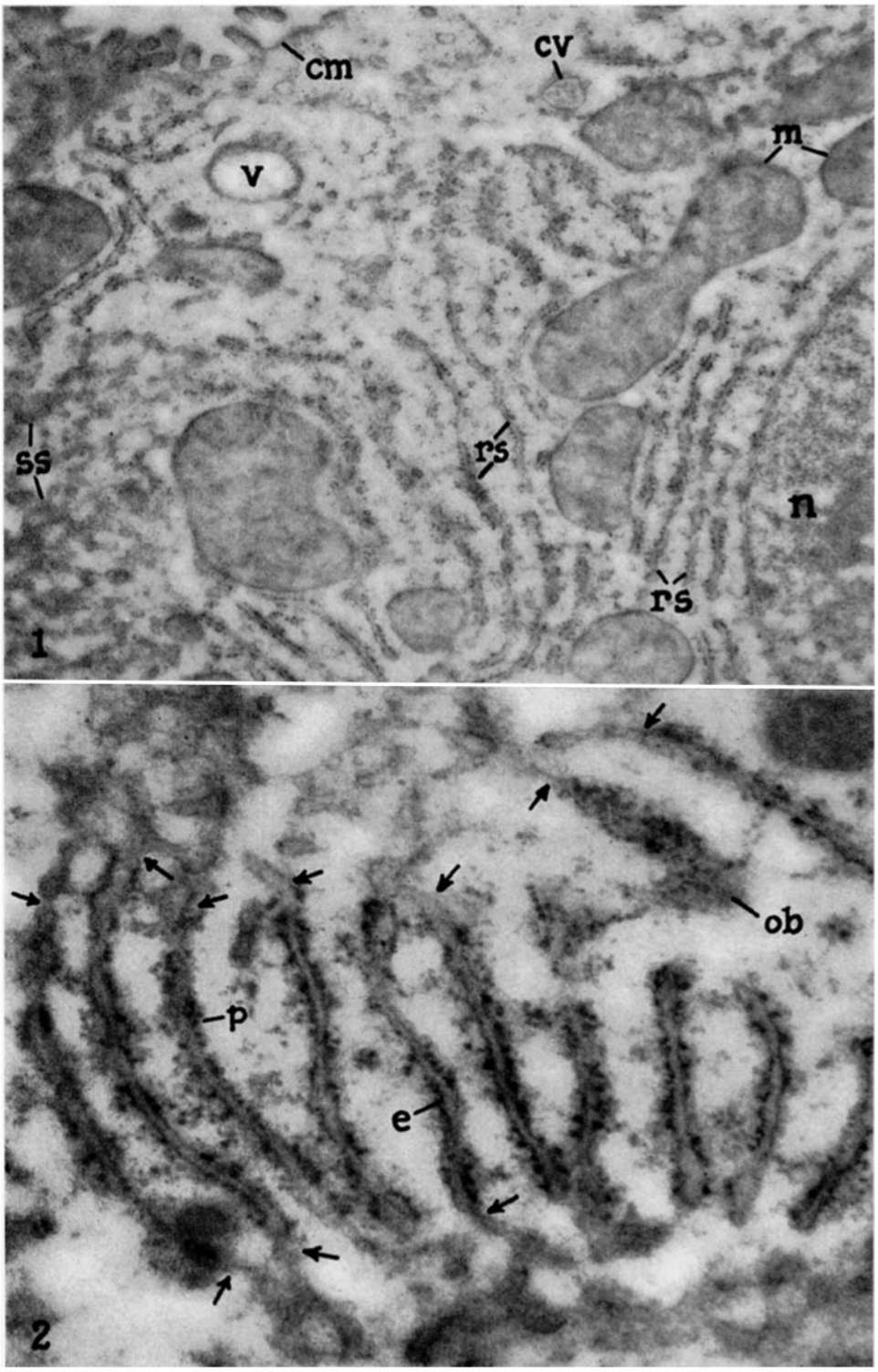
PLATE 28

FIG. 1. The electron micrograph shows part of a parenchymatous liver cell at a relatively low magnification.

The nucleus appears at *n* and the cell membrane, limiting a bile capillary, at *cm*. A number of mitochondrial profiles (*m*) can be seen in the cytoplasm that contains, in addition, numerous profiles of the endoplasmic reticulum. The latter vary in shape from circular to elongated and belong to both the smooth surfaced (*ss*) and the rough surfaced variety (*rs*). The vacuolated structure at *v* probably derives from a dense, peribiliary body. At *cv* appears a compound vesicle of the type already described in perikarya. × 26,000.

FIG. 2. This small field in the cytoplasm of a parenchymatous liver cell shows an array of 10 elongated profiles, one of them marked *e*, which in 3 dimensions correspond to a pile of preferentially oriented cisternae. The profiles belong to the rough surfaced variety; *i.e.* bear small, dense particles (*p*) attached to the outer surface of their limiting membrane. The normally sectioned profiles show clearly their limiting membrane and their content. An obliquely sectioned cisterna (*ob*) offers a side view of its particle-dotted membrane.

The upper left and the lower right corners of the figure are occupied by short, contorted profiles of smooth surfaced variety. Their distribution indicates that in 3 dimensions they correspond to a tightly meshed, randomly oriented reticulum. Rough surfaced and smooth surfaced profiles are found in continuity in many places (arrows), a finding that indicates that the two profile varieties are not different, unrelated structures, but represent local differentiations within a common, continuous system, the endoplasmic reticulum. × 63,000.



(Palade and Siekevitz: Liver microsomes)

PLATE 29

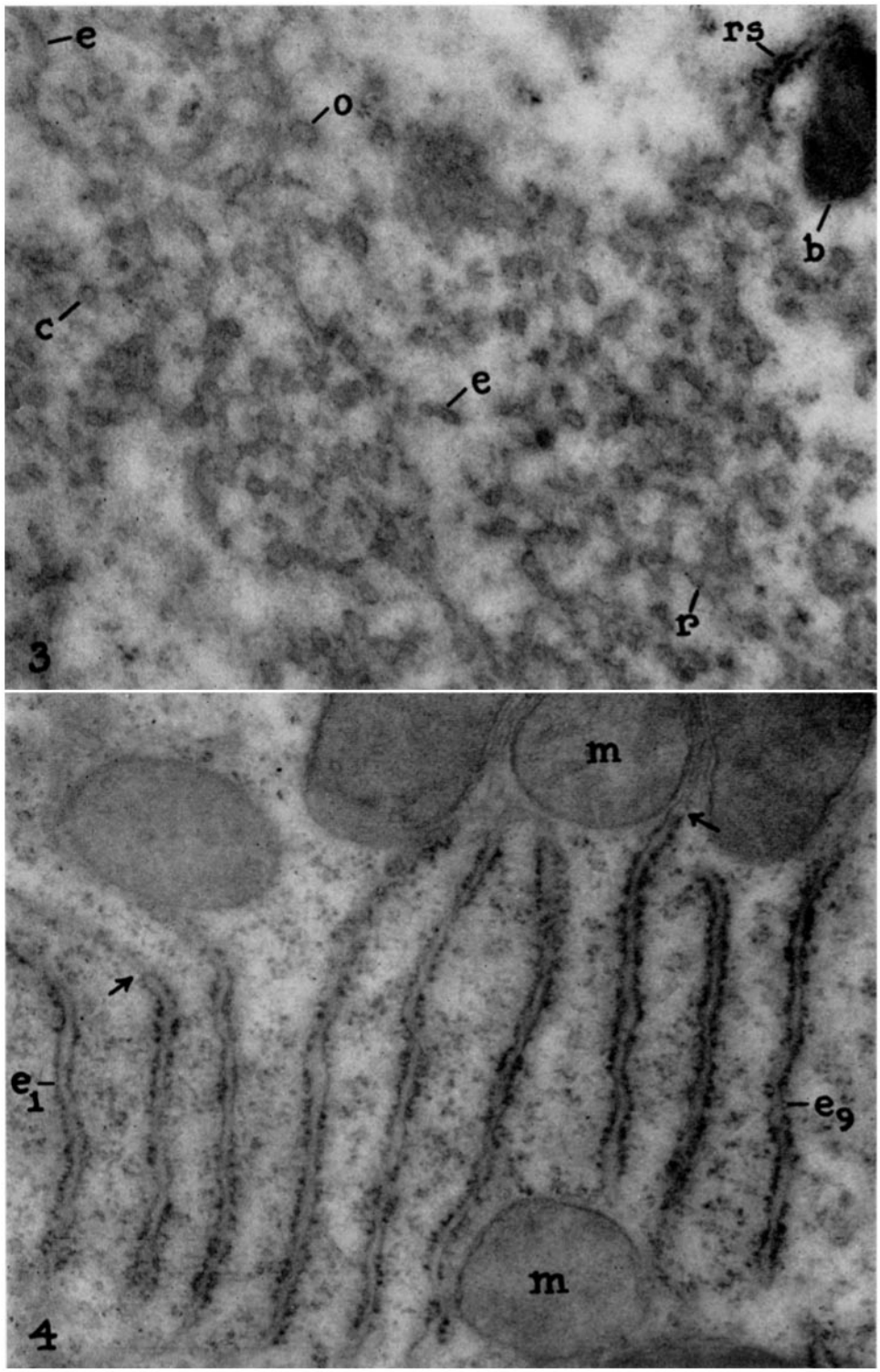
FIG. 3. Small field in the peripheral cytoplasm of a parenchymatous liver cell.

The field is taken by numerous circular (*c*), oval (*o*), and short, oblong (*e*) profiles of smooth surfaced variety. They are disposed at random and characteristically grouped in tightly packed masses. In 3 dimensions they correspond to a tightly meshed, randomly disposed network. In the lower right corner of the figure a few almost complete meshes (*r*) are included in the thickness of the section.

A rough surfaced profile appears at *rs*, and a dense peribiliary body at *b*. × 48,000.

FIG. 4. The figure shows an array of nine elongated profiles (e_1, e_9) of the rough surfaced variety and five mitochondrial profiles (*m*) in the cytoplasm of a parenchymatous liver cell. The elongated profiles are disposed parallel to one another at more or less regular intervals. In 3 dimensions, the array corresponds to a pile of cisternae. The outside surface of the membrane limiting these profiles bears numerous attached particles of small size and high density. A few similar particles appear freely scattered in the intervening cytoplasm.

The arrows indicate continuity between rough surfaced and smooth surfaced profiles. × 49,000.



(Palade and Siekevitz: Liver microsomes)

PLATE 30

FIG. 5. Small field in the peripheral cytoplasm of a parenchymatous liver cell.

The cell membrane can be seen in the lower part of the figure outlining a bile capillary (*bc*). The profiles of three dense bodies (b_1, b_2, b_3) appear in the vicinity of the capillary. Such bodies possess, in general, a limiting membrane and a dense, remarkably polymorphic content. A granular texture of fine or coarse grain is the usual occurrence but bodies containing lumps of dense material or small vacuoles with a thick or thin wall are also frequently encountered. The fine grains are sometimes unusually dense, suggesting the presence of a metal compound. In their general morphology these dense peribiliary bodies are reminiscent of the residual bodies found in microphages.

At *cs* appears an agglomeration of tightly packed, smooth surfaced profiles, similar to the ones described in the centrosphere region of other cells and identified as the Golgi apparatus by certain workers. Vacuoles containing small vesicles or grains can be seen within this agglomeration. × 46,000.

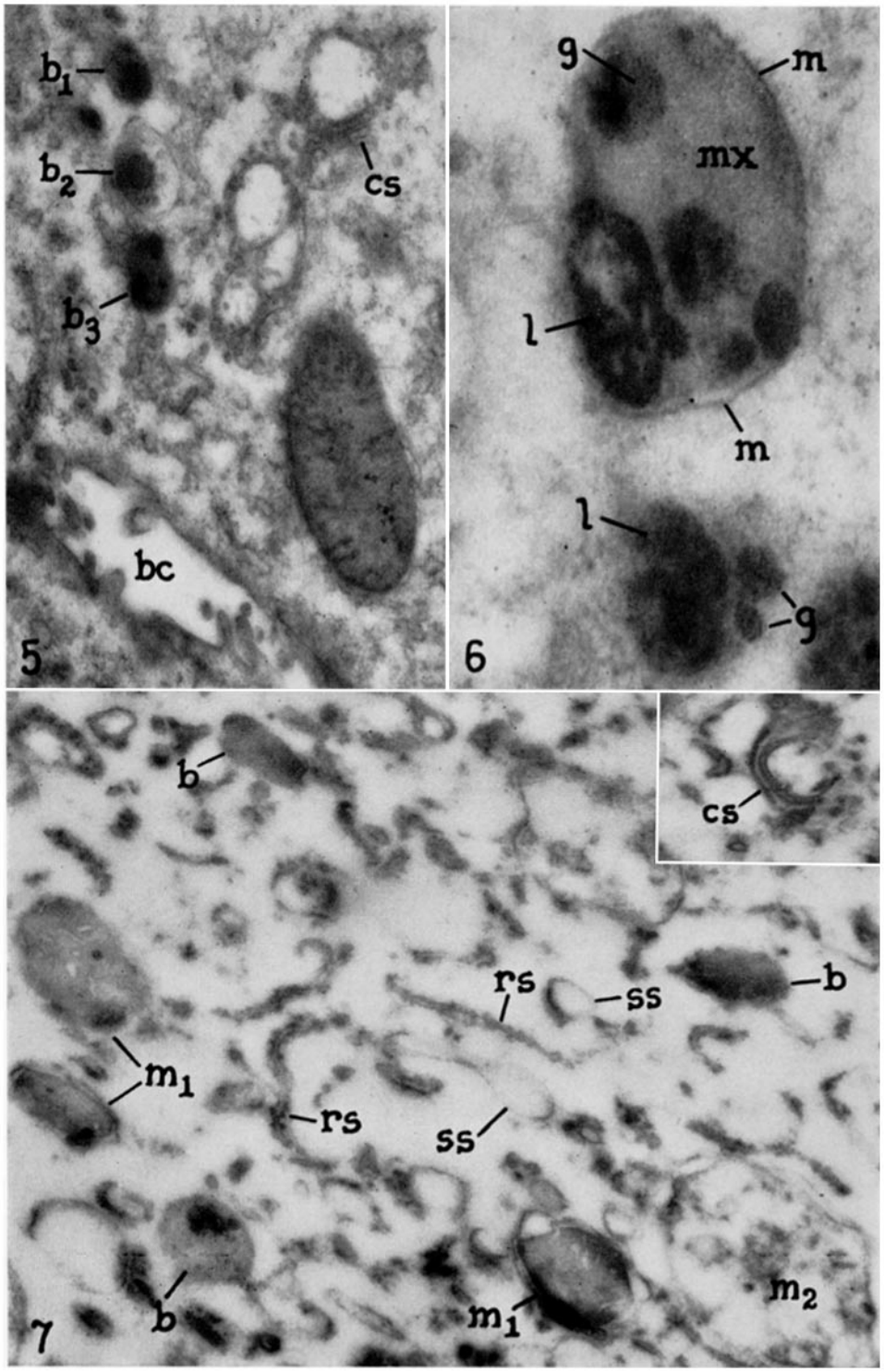
FIG. 6. The figure shows at a high magnification the profiles of two dense, peribiliary bodies.

A limiting membrane (*m*) can be seen around most of the outline of the upper profile. The bodies contain irregular lumps (*l*) of dense material embedded in a less dense matrix (*mx*). Small granules of high density (*g*) are frequently present within the dense lumps. × 127,000.

FIG. 7. The figure shows a relatively small field in a section through a pellet obtained from a liver homogenate. After fixation in 2 per cent OsO_4 in 0.88 M sucrose (24 hours at 0°C.), the homogenate was centrifuged for 30 minutes at 105,000 *g* and the pellet thus obtained was dehydrated and embedded *in toto*. The field in this figure was located at the merging of the mitochondrial and microsomal layers of the pellet.

Apparently intact mitochondria appear at m_1; another mitochondrion, damaged, swollen and extracted, at m_2; and dense, peribiliary bodies at *b*.

Fragments of the endoplasmic reticulum can be seen scattered all over the field. Most of them belong to the rough surfaced variety (*rs*) as indicated by the small, dense particles still attached to their membranes. Smooth surfaced profiles (*ss*) may derive either from the smooth surfaced parts of the endoplasmic reticulum or from the piles of membranous structures marked *cs* in the insert. The insert shows an array of tightly packed, smooth surfaced profiles found in another section of the same pellet. The array corresponds probably to the peribiliary structures identified by Fawcett (47) as the Golgi apparatus of the parenchymatous liver cell. Usually these membranous elements are dispersed during homogenization and only occasionally retain their characteristically tight packing. × 33,000.



(Palade and Siekevitz: Liver microsomes)

PLATE 31

FIG. 8. A relatively large, representative field in a section of microsome pellet fixed *in toto* in 2 per cent OsO_4 in 0.88 M sucrose (24 hours at 0°C.). At first glance the fraction appears rather unhomogeneous, but closer examination shows that the pellet consists mainly of fragments of the rough surfaced parts of the endoplasmic reticulum, most of the apparent diversity being explained by differences in the angle of cutting. When normally sectioned, the rough surfaced fragments (*n*) clearly show their limiting membrane, with attached dense particles on its outer aspect, and their content which is slightly denser than the embedding plastic. The cavity of obliquely sectioned elements (ob_1) is obscured to a variable extent. Very obliquely sectioned elements (ob_2) offer a full faced view of their membrane and of the particles attached thereon.

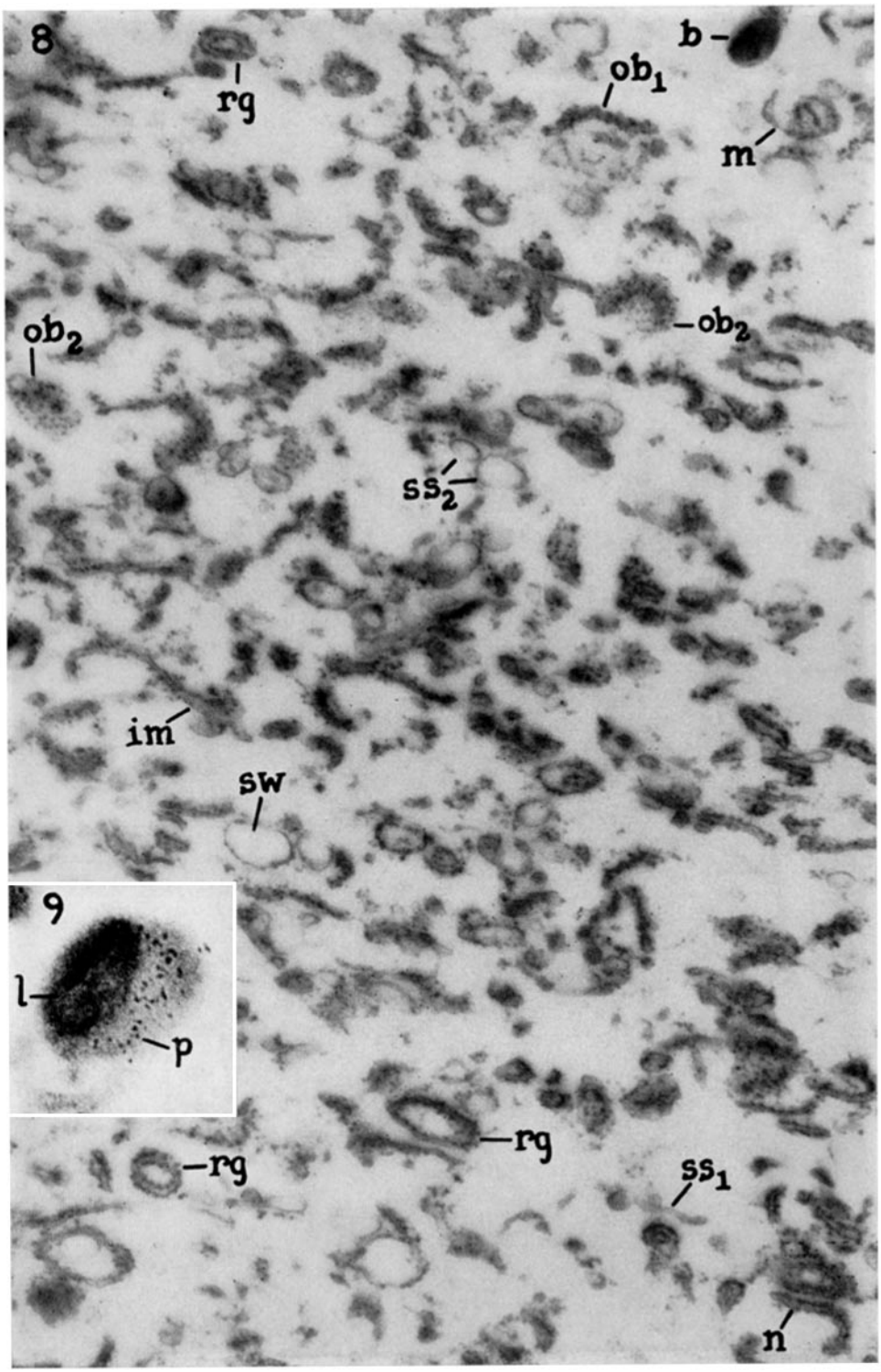
Note that the fragments of the endoplasmic reticulum have closed ends; that some of them are of considerable length; that small cisternae are frequent among them (only cisternal elements could give the appearances marked ob_2); and that they retain the flattened form, characteristic for intracellular cisternae. Relatively few rough surfaced elements appear to be swollen (*sw*). Note also the frequency of ring-shaped profiles (*rg*) bearing attached particles. As such formations are extremely rare in cells *in situ*, it can be assumed that they are the result of the rolling and fusion of cisternal fragments during homogenization.

Smooth surfaced profiles (ss_1) are relatively few. They are frequently larger (ss_2) than corresponding profiles *in situ*. The increase in size may be due to swelling. These profiles may derive either from the smooth surfaced parts of the reticulum or from the piles of peribiliary cisternae (Golgi apparatus). "Intermediate forms," partly covered and partly free of particles (*im*), are occasionally encountered.

In addition to the elements described, the microsome pellets contain a few, dense, peribiliary bodies (*b*) and occasionally fragmented and extracted mitochondria (*m*). × 44,000.

FIG. 9. A dense peribiliary body of the same microsome pellet at a higher magnification.

There is no visible membrane around this body which contains a relatively large, dense lump (*l*) and a collection of small, very dense particles (*p*), ~40 to 80 A in diameter, all embedded in an apparently homogeneous matrix. × 127,000.



(Palade and Siekevitz: Liver microsomes)

PLATE 32

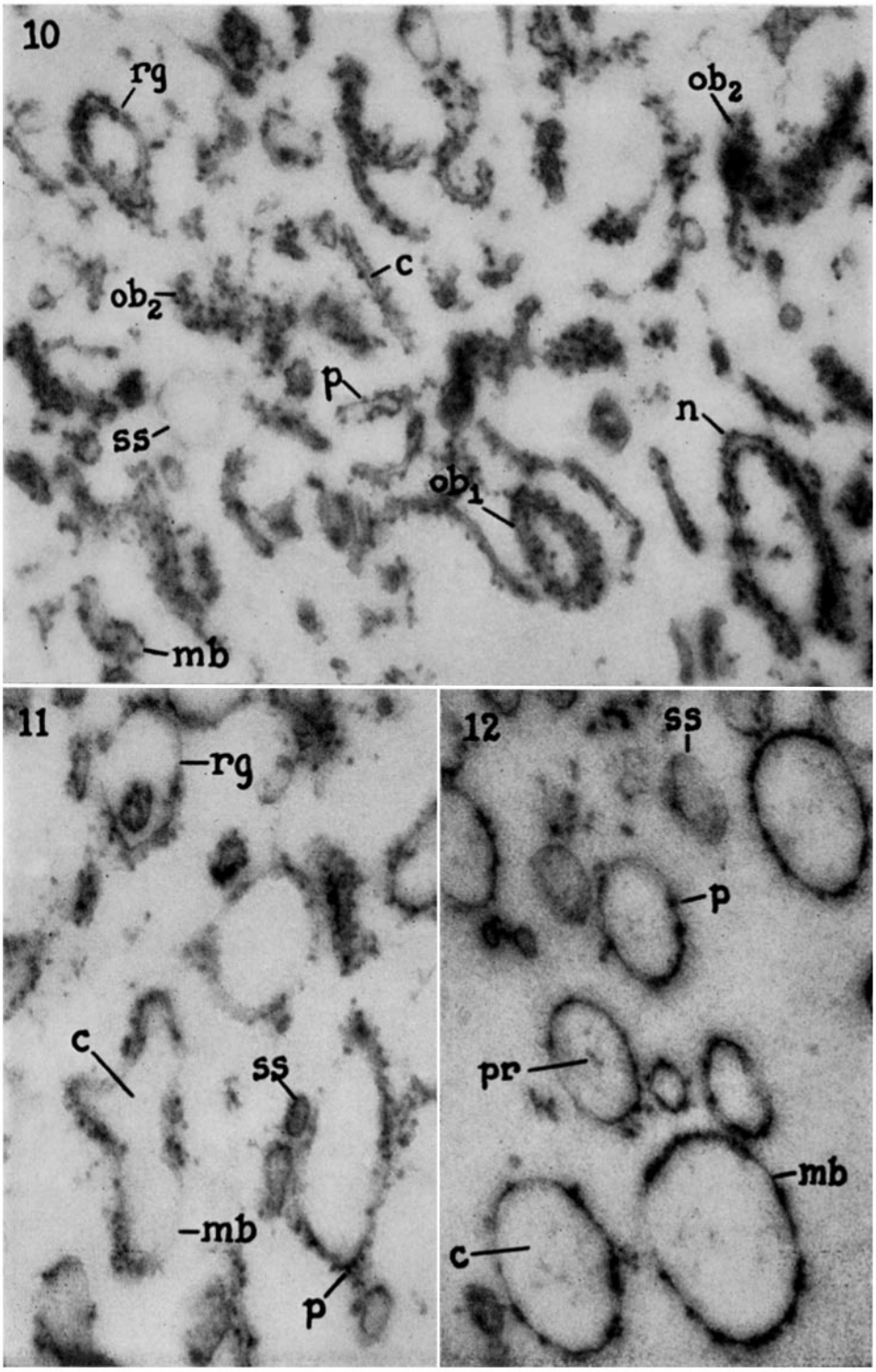
FIGS. 10 to 12. These figures illustrate the osmometer-like behavior of isolated microsomes. All corresponding microsome pellets were isolated from liver homogenates prepared in 0.88 M sucrose. All were fixed *in toto* in 2 per cent OsO_4 dissolved in 0.88 M sucrose for the pellet in Fig. 10, in 0.028 M Na acetate—0.028 M Na veronal for the one in Fig. 11 and, finally, in distilled H_2O for the pellet in Fig. 12.

FIG. 10. The electron micrograph shows that microsomes fixed in the presence of 0.88 M sucrose retain the characteristic, flattened appearance of intracellular cisternae and appear to have a content slightly denser than the embedding plastic.

FIG. 11. The microsomes appear as relatively large vesicles with a content similar in density to the embedding plastic. A comparison with Fig. 10 indicates that these microsomes have swollen in the hypotonic fixative without losing the small, dense particles attached to their membrane.

FIG. 12. Fixation in the presence of distilled water has caused further increase in size and complete enspherulation of the microsome vesicles (their oval appearance is due to compression by the microtome knife). They retain their attached particles, but the latter appear less distinct than in Fig. 10. Some of the microsomal vesicles contain a faint, fine precipitate (*pr*). In all three figures, *mb* indicates the membrane of the microsomes; *c*, their content; *p*, attached particles; and *ss*, smooth surfaced profiles.

For Figs. 10 and 11, *rg* indicates ring-shaped profiles. In Fig. 10, *n* is a normally sectioned fragment of the endoplasmic reticulum, ob_1 and ob_2, fragments cut at increasing degrees of obliquity. All three figures × 60,000.



(Palade and Siekevitz: Liver microsomes)

PLATE 33

FIGS. 13 and 14. Pellets of a first postmicrosomal fraction obtained by centrifuging the microsomal supernatant for 3 hours at 105,000 *g*. Both pellets were fixed *in toto* in 2 per cent OsO_4 dissolved in an "isotonic" salt solution for the pellet in Fig. 13, and in 0.88 M sucrose for the one in Fig. 14.

The micrographs show that this fraction consists mainly of smooth surfaced membranous elements (*ss*), a few, apparently free particles (*p*), and a certain amount of amorphous material. Rough surfaced elements (*rs*) are very rare or absent. A comparison of the two micrographs shows that the smooth surfaced elements behave like osmometers: they appear as predominantly elongated profiles with a dense content when fixed in the presence of 0.88 sucrose (Fig. 14), and as larger oval or circular profiles after fixation in "isotonic" media (Fig. 13). These elements may derive either from the smooth surfaced parts of the endoplasmic reticulum or from the piles of peribiliary cisternae (Golgi apparatus).

FIG. 15. Pellet of a second postmicrosomal fraction (obtained by centrifuging the supernatant of the preceding fraction for 15 hours at 105,000 *g*) fixed *in toto* in 2 per cent OsO_4 dissolved in 0.88 M sucrose.

The fraction consists mainly of an apparently amorphous material in which are embedded a few smooth surfaced vesicles (*ss*) and occasionally a few isolated, small, dense particles. Figs. 13 to 15, × 40,000.

FIG. 16. Microsomes treated with 2 per cent versene. Pellet fixed *in toto* in 2 per cent OsO_4 in 0.88 M sucrose.

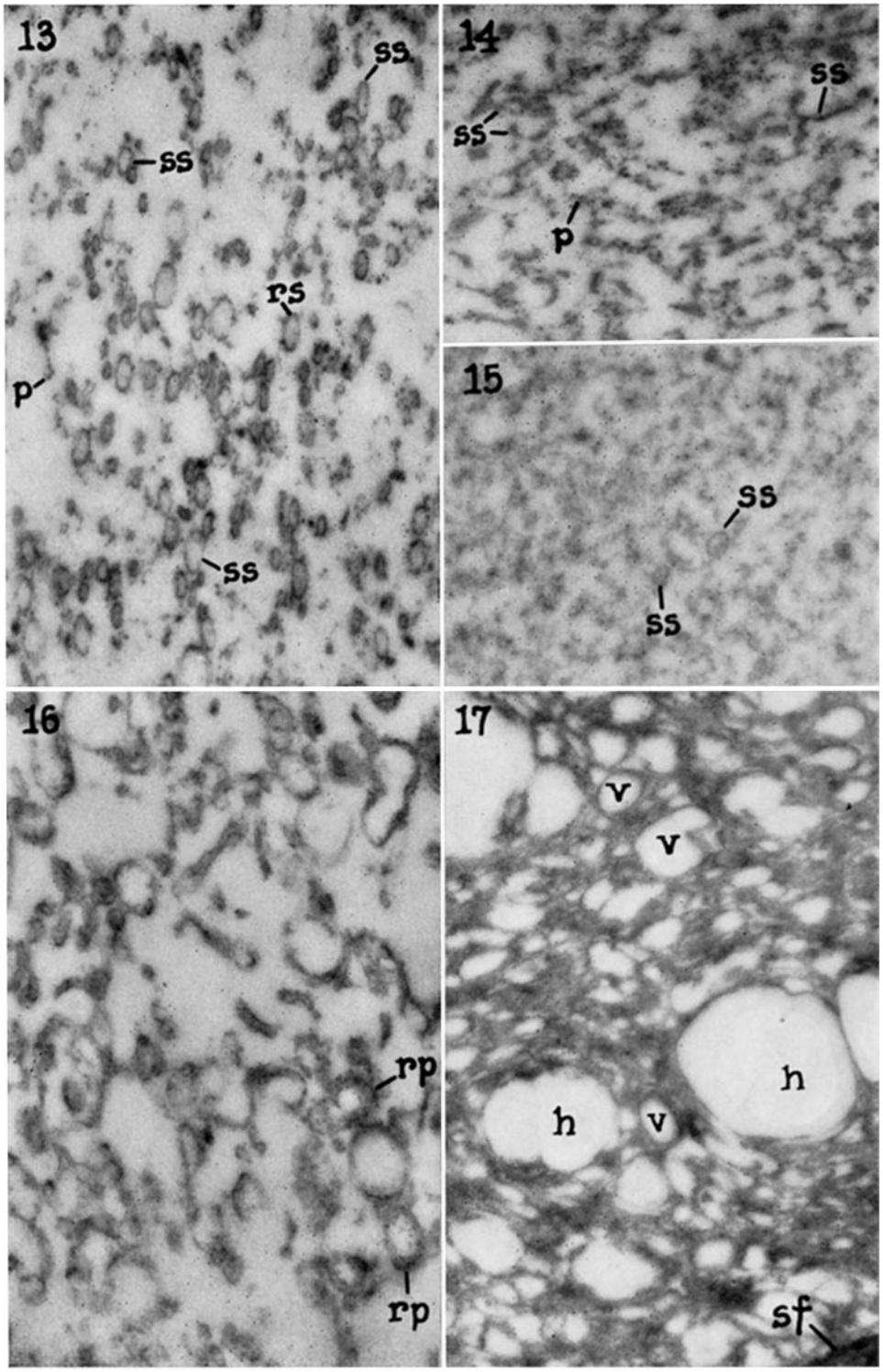
Although some fragmentation and swelling have taken place, most microsomes have retained their flattened appearance. They have lost, however, their attached small particles. Only a few remnants can be seen on the elements marked *rp*. × 40,000.

FIG. 17. Microsomes incubated for 30 minutes at 37°C. in the presence of ribonuclease (5 mg./ml.).

Pellet fixed *in toto* in 2 per cent OsO_4 in 0.88 M sucrose.

Note the heavy agglutination and the pronounced swelling undergone by the microsomes as a result of this treatment. Profiles of individual vesicles can be seen at *v*; their membrane appears to be smooth, without visible, attached particles.

The section was micrographed through a "window" in the supporting film. The rim of the window can be seen at *sf*. Because of direct exposure to the electron beam, holes (*h*) have appeared in the plastic filling some of the swollen, microsomal vesicles. × 40,000.

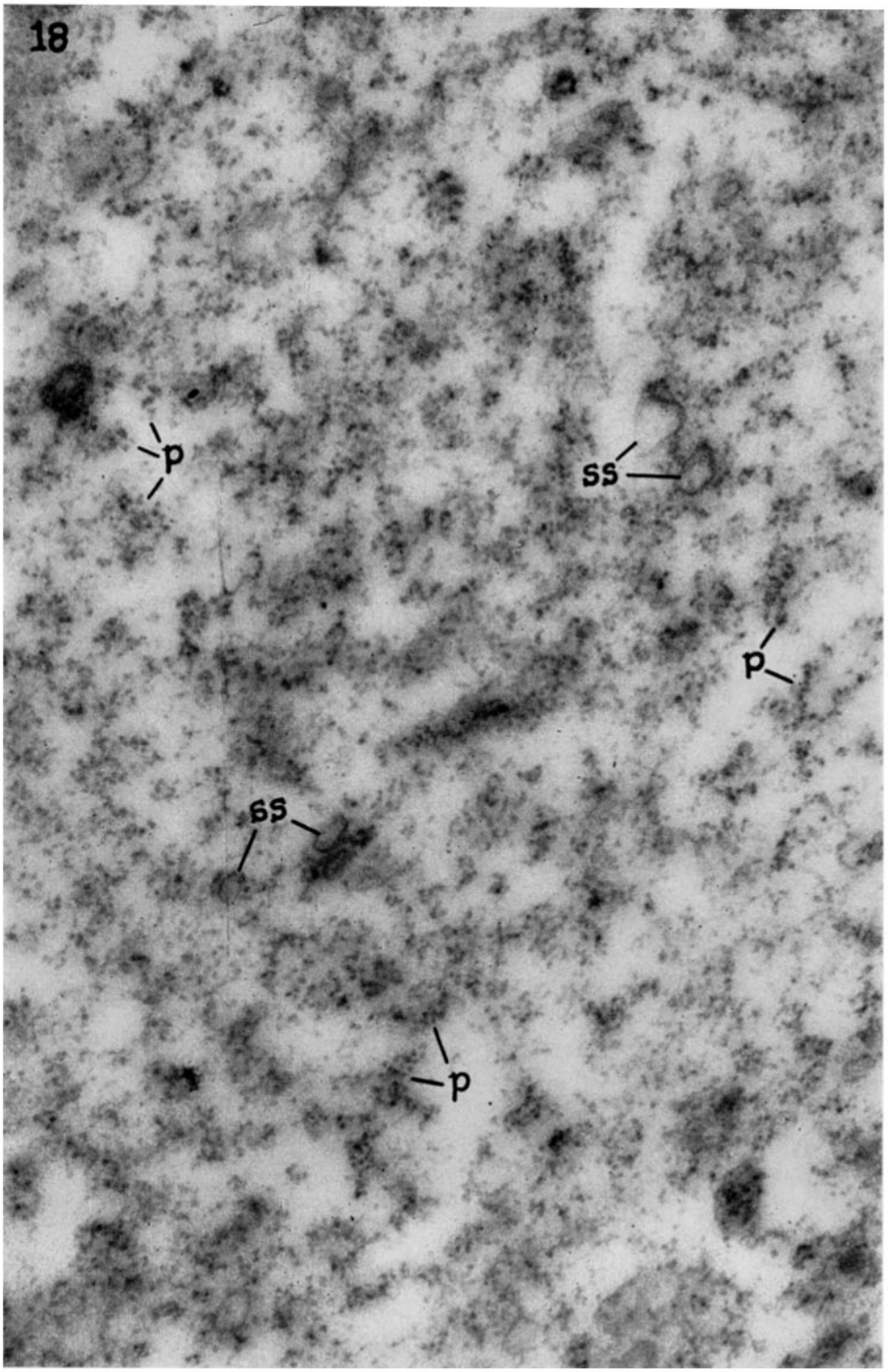


(Palade and Siekevitz: Liver microsomes)

PLATE 34

FIG. 18. Microsomes treated with 0.5 per cent Na deoxycholate. Pellet fixed *in toto* in 2 per cent OsO_4 in 0.15 M NaCl (18 hours at 0°C.).

The micrograph shows a relatively large, representative field in the pellet which appears to consist mainly of small, dense particles (*p*), 100 to 150 A in diameter. In shape, size, and density they are similar to the particles attached to the outside surface of the limiting membrane of untreated microsomes. The membranous component of the microsome fraction has been almost completely "solubilized" by the deoxycholate treatment. The few smooth surfaced vesicles that remain (*ss*) appear swollen as a result of the low tonicity of the fixative and thus are more easily visualized. × 55,000.



(Palade and Siekevitz: Liver microsomes)