MORPHOLOGICAL AND BIOCHEMICAL CORRELATES OF CEREBRAL MICROSOMES

I. Isolation and Chemical Characterization

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ABSTRACT

Microsomal fractions, both homogeneous in appearance and functionally operative, were isolated from a homogenate of rat cerebral cortex by fractionation in water. The preparations thus obtained contain the membranous elements of the endoplasmic reticulum, synaptic vesicles, and ribosomes. Esterase, ATPase, and glutamine synthetase were found to be present and fully functional in the microsomal fractions isolated in water. The contamination of the water-isolated microsomal fractions by mitochondria and lysosomes was found to be considerably lower than in microsomal pellets isolated in sucrose. The contamination by nerve ending particles, as judged by electron microscopy and by the levels of soluble lactic dehydrogenase entrapped in the cytoplasm of the particles, was also low. Most of the contamination by mitochondria and nerve ending particles could be removed by treatment of the microsomal pellet with 150 mM NaCl. Resistant to elution by this treatment is the lysosomal contamination as well as microsomal esterase and ATPase. Glutamine synthetase, on the other hand, was almost totally solubilized. Microsomal preparations isolated in water are also shown to contain amounts of protein, RNA, phospholipid, and ganglioside comparable to those found in microsomal preparations isolated in sucrose.

The microsomal fraction isolated from mammalian tissues by high speed centrifugation has not been unequivocally characterized, for it has often been found to consist of disrupted fragments of intracellular materials, including the cellular envelope, that connot readily be compared with, or identified with, intact structures (1, 2). This is particularly true for fractions isolated from brain, owing to the complex and heterogeneous organization of this organ. In contrast to brain is the liver, for instance, from which fractions can be isolated which are much more homogeneous as a consequence of the less complex organization of this organ. Palade and Siekevitz (3) have demonstrated that in rat liver the microsomal fraction consists preponderantly of vesicular and tubular fragments of the intracellular endoplasmic reticulum and of ribosomal granules. Hanzon and Toschi (4) described a similar composition for the microsomal fraction of brain tissue. However, more recent studies by de Robertis *et al.* (5) and by Whittaker (6) have revealed that the microsomal fraction of brain, as usually isolated in sucrose media, consists of a rather heterogeneous popula-

tion of components, including small myelin fragments, lysosome-like structures, nerve ending particles, synaptic vesicles, and occasional small mitochondria.

Subfractionation of the microsomal fraction of brain by both density gradient centrifugation (7–9) and phase partitioning in aqueous media (10) has also been reported. These studies have been mainly directed toward the isolation of ribosomal fractions (4, 11–13). To our knowledge, however, no studies have been reported in which the effect of the centrifugation medium upon the chemical and structural integrity of the microsomal fraction has been critically evaluated, presumably because the assumption has prevailed that the osmotic protection required for maintenance of structural integrity of mitochondria and lysosomes is also necessary to preserve microsomal integrity.

The differential behavior of two cerebral enzymes, glutamine synthetase and esterase (substrate, *o*-nitrophenyl acetate), toward homogenization and centrifugation in increasing concentrations of sucrose has been described previously (14). These studies showed that the maximal degree of attachment of glutamine synthetase to the membranes of the microsomal fraction was obtained when sucrose-free media were used for the isolated of the fraction. Recently de Robertis *et al.* (15, 16) and Whittaker *et al.* (17, 18) have subjected cerebral fractions containing nerve ending particles to osmotic shock by suspension in distilled water. This has led to the isolation of relatively clean preparations of synaptic vesicles.

In this report, further evidence is presented to show that, when examined under the electron microscope, microsomal pellets isolated from rat cerebral cortex by centrifugation in water exhibit a more homogeneous appearance than pellets obtained by centrifugation in sucrose. Also, isolation by centrifugation in water, as ascertained by chemical and enzymic analysis, leads to a microsomal preparation with a composition compatible with several known microsomal functions (19).

EXPERIMENTAL PROCEDURE

Materials

Osmium tetroxide was purchased from Merck and Co. (Darmstadt, West Germany). Maraglas used for embedding the pellets to be examined by electron microscopy was obtained from Marblette Research Laboratory, Long Island City, New York. Malic dehydrogenase was a suspension in ammonium sulfate of the swine heart enzyme (Worthington Biochemical Co., Freehold, New Jersey).

Methods

ENZYME ASSAYS: Cytochrome oxidase was determined by the method of Cooperstein and Lazarow (20) as modified by Appelmans *et al.* (21). The activity is expressed as Δ A (550 mμ) per 20 seconds.

Aspartic transaminase was determined by the spectrophotometric method of Karmen (22) as modified for cerebral tissue by Sellinger and Rucker (23). One unit of activity refers to the oxidation of 1 μmole of NADH per hour.

Acid phosphatase was determined as described by Gianetto and de Duve (24). Unless otherwise stated, the incubation period was 3 hours. The activity is expressed as micromoles of inorganic phosphate liberated per hour.

Glutamine synthetase was determined as described previously (14). One unit of activity refers to the amount of enzyme required to produce 1 μmole of L-glutamylhydroxamate per hour.

Esterase was determined as described by Sellinger and de Balbian Verster (25), using *o*-nitrophenyl acetate as substrate. One unit of enzyme refers to the amount required to liberate 1 μmole of *o*-nitrophenol per 30 minutes.

Microsomal ATPase was determined according to Jarnefelt (26). The inorganic phosphate liberated in the reaction (10 minutes at 37°C) was determined by the method of Martin and Doty (27). The units are micromoles of inorganic phosphate liberated per minute.

Lactic dehydrogenase was assayed spectrophotometrically by the method of Johnson (28) with lithium pyruvate as substrate. The activity is expressed as Δ A (340 mμ) per 100 seconds.

CHEMICAL DETERMINATIONS: Protein was determined by the method of Lowry *et al.* (29). RNA was determined as previously described (14) Microsomal phospholipids were extracted by the method of Folch *et al.* (30). The lower phase resulting after the addition of 0.1 M KCl to the chloroform-methanol extract was taken to dryness under nitrogen, and the residue was digested with 60 per cent (*v/v*) $HClO_4$, after which inorganic phosphate was determined (27). Microsomal gangliosides were estimated by subjecting the upper phase of the chloroform-methanol extract, previously evaporated to dryness under nitrogen, to a 2-hour period of hydrolysis at 80°C in 0.2 ml of 0.1 N sulfuric acid. The *N*-acetyl neuraminic acid content of the hydrolyzate was determined by the method of Warren (31).

PREPARATION OF THE FRACTIONS: Male Holtzman rats weighing between 150 and 250 grams

were decapitated, and the brain was removed and blotted. The cortex was ablated and placed in a tared beaker containing ice cold centrifugation medium (0.25 M sucrose or distilled water). The entire procedure was accomplished in less than 30 seconds. The tissue was homogenized in approximately 4 volumes of medium as previously described (14). The homogenate was centrifuged for 10 minutes at 25,000 *g*. The resulting sediments (fraction NML) were resuspended in the respective media for isolation and were centrifuged for an additional 10 minutes at 25,000 *g*. The supernatants (fraction PS) were combined, diluted to give a 1 to 10 suspension, and centrifuged at 145,000 *g* for 60 minutes to yield a microsomal pellet (fraction P) and a supernatant (fraction S).

In some cases the above "slow" procedure was replaced by a "fast" procedure, in which fraction NML was not washed and the supernatant from the first centrifugation step was diluted directly to give a 10 per cent suspension. The pellets and supernatant fluids obtained by either procedure were subsequently treated in an identical fashion.

For chemical and enzymic analysis, all pellets were resuspended in the media used for their isolation. In cases where the effect of compounds on the pellet (fraction P) isolated in water was to be investigated, the pellet was resuspended by manual homogenization to ensure complete dispersion. This suspension (fraction PS_1) was then centrifuged as before to yield a pellet (P_1) and a supernatant (S_1).

For electron microscopy, the high speed supernatant (fraction S) was decanted and the cellulose nitrate (Spinco Division, Beckman Co., Palo Alto, California) tube was cut away around the microsomal pellet (P), care being taken to keep the pellet on ice. Samples were removed from the top, middle, and bottom portions of the pellet and were placed in freshly prepared ice cold 1 per cent (*w/v*) osmium tetroxide. The latter was made up in 0.25 M sucrose or in water. After fixation (1 hour at 0°C), the samples were removed and were serially dehydrated in 10 per cent gradations of ice cold ethanols at 10-minute intervals. The specimens were embedded in Maraglas according to Freeman and Spurlock (32). The ultrathin sections, cut on a Porter-Blum microtome, were lead stained according to Dalton and Zeigel (33 and were examined in a Siemens Elmiskop I.

RESULTS

Morphological Findings

ISOLATION IN 0.25 M SUCROSE BY THE FAST PROCEDURE: Samples obtained from three different layers in the pellets obtained by centrifugation in sucrose by the fast procedure (see Methods) were markedly different in appearance. The layers differed mainly in the amount of mitochondrial and myelin contamination, the bottom layer being most grossly contaminated with non-microsomal material.

A survey of the pellets in a plane parallel to the centrifugal field showed that the layers consisted of a variety of structures, many of the vesicles having a crenated appearance. A characteristic area from the middle layer is shown in Fig. 1. In addition to structures which appear to be derived from the endoplasmic reticulum, there were included nerve ending particles, mitochondria, and many structures of unknown origin.

ISOLATION IN 0.25 M SUCROSE BY THE SLOW PROCEDURE: The interposition of an additional step to extract the NML fraction led to a microsomal pellet which appeared to be less contaminated. Although there still were differences in appearance among the samples obtained from the three different layers examined, the differences were less marked than in comparable layers of pellets obtained by the fast procedure. A survey of the layers in a plane parallel to the centrifugal field showed again a rather heterogeneous field. The middle layer shown in Fig. 2, although "cleaner" than the same layer in the pellet obtained by the fast procedure, still contained many structures that were non-microsomal in appearance.

ISOLATION IN WATER BY THE FAST PROCEDURE: Microsomal pellets isolated in water without washing of the NML fraction showed some differences in the content of the three layers examined. The top and middle layers (Fig. 3) contained many ribosomal clusters, while the bottom layer contained a preponderance of smooth vesicles and few ribosomal clusters. The layers were characterized by their homogeneous appearance. The fields consisted almost entirely of ribosomes, large vesicles mostly ranging between 0.1 and 0.2 μ, and small vesicles mostly 0.04 μ in diameter. The latter vesicles, as judged by their size and appearance, very likely represent synaptic vesicles liberated from the nerve ending particles owing to exposure to the hypotonic medium.

ISOLATION IN WATER BY THE SLOW PROCEDURE: Examination of the pellet obtained by the slow procedure in water revealed, in contrast to the pellet obtained in sucrose, very homogeneous fields throughout the entire pellet. There appeared to be a preponderance of vesicles

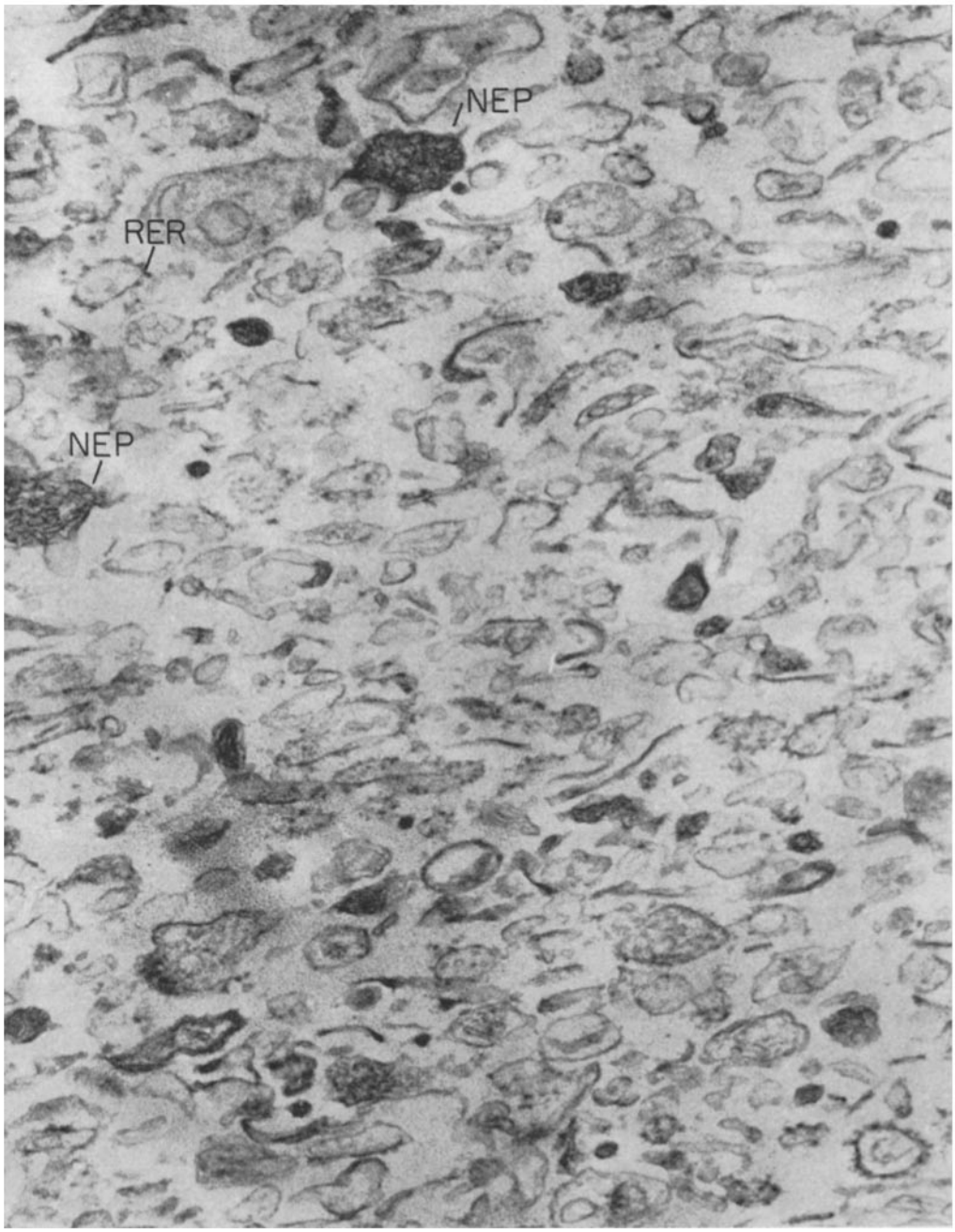

FIGURE 1 Electron micrograph of microsomal pellet isolated by high speed centrifugation of rat cerebral cortex homogenate in 0.25 M sucrose. Micrograph represents middle layer of pellet obtained by the "fast" procedure. *NEP*, nerve ending particle; *RER*, rough endoplasmic reticulum. × 45,000.

of probable ER origin, some with ribosomes attached. The smaller vesicles, presumably synaptic vesicles, were present in a relatively smaller amount than in the pellet isolated by the fast procedure. In addition, only very few components obviously not ER in origin, other than synaptic vesicles, were observed to be included in the pellet (Fig. 4).

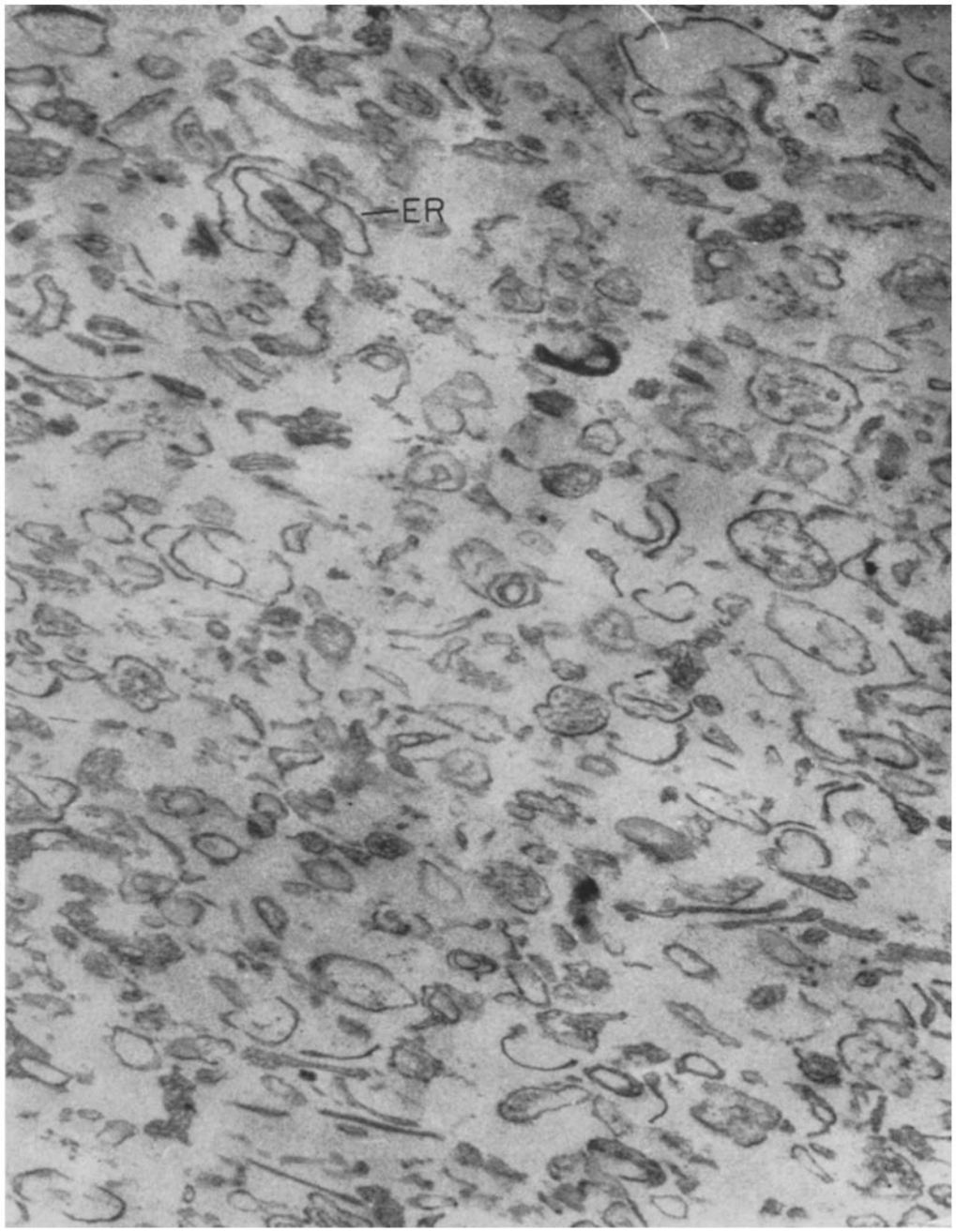

FIGURE 2 Electron micrograph of microsomal pellet isolated by high speed centrifugation of rat cerebral cortex homogenate in 0.25 M sucrose. Micrograph represents middle layer of pellet obtained by the "slow" procedure. *ER*, endoplasmic reticulum. × 45,000.

Chemical Findings

The relative distribution obtained for four "marker" enzymes upon differential centrifugation of cerebral homogenates in either water or 0.25 M sucrose is shown in Table I. The mitochondrial marker, cytochrome oxidase, was distributed almost exclusively in the NML fraction, irrespective of the centrifugation medium used.

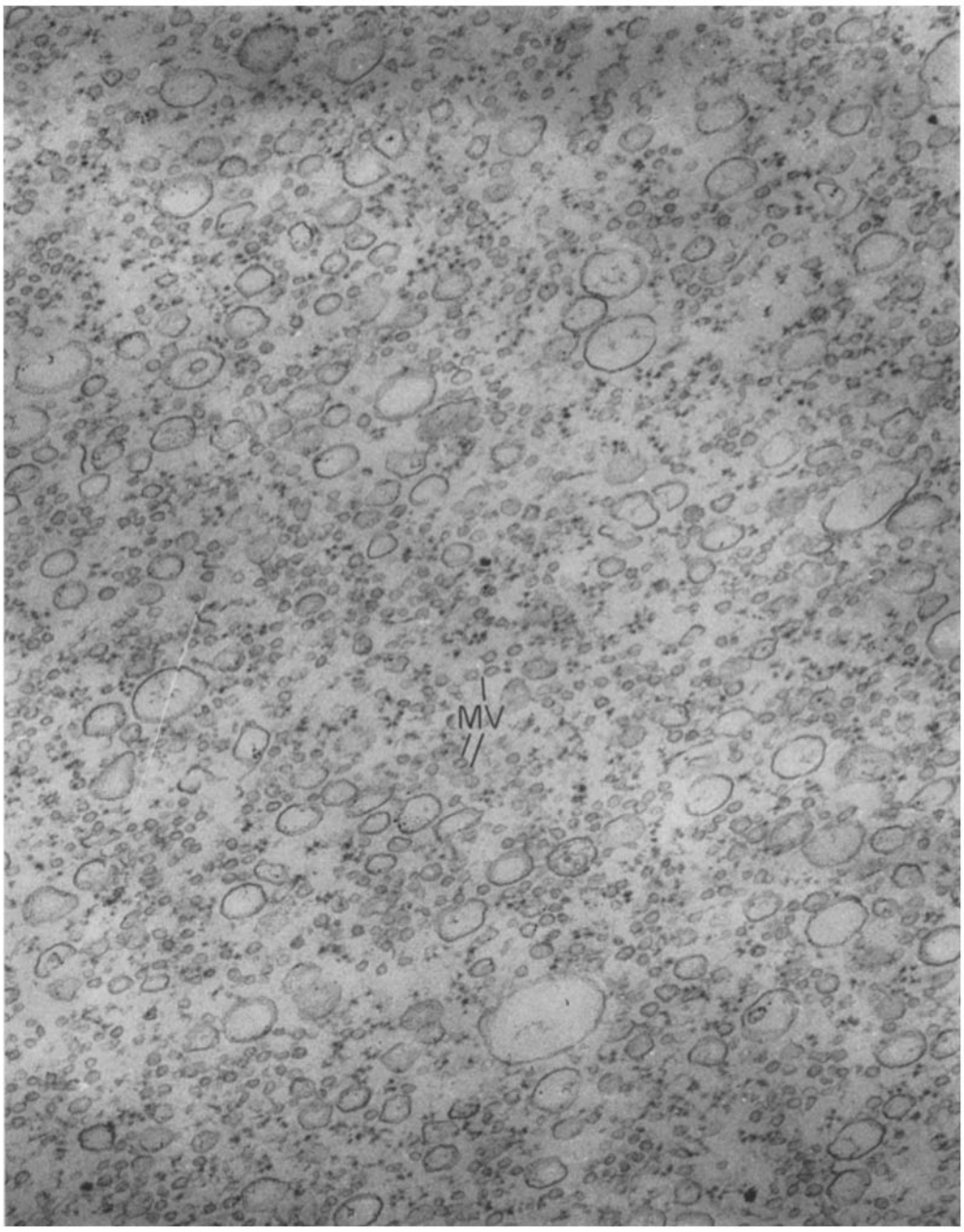

FIGURE 3 Electron micrograph of microsomal pellet isolated by high speed centrifugation of rat cerebral cortex homogenate in water. Micrograph represents middle layer of pellet obtained by the "fast" procedure. *MV*, microvesicles. × 45,000.

The localization of the lysosomal marker, acid phosphatase, shifted from the NML fraction to the supernatant in the preparation fractionated in water. It may be noted that there was much less solubilization of the lysosomes in the sucrose medium, *i.e.*, the sucrose supernatant contained less acid phosphatase than the water supernatant; correspondingly, the microsomal pellet isolated

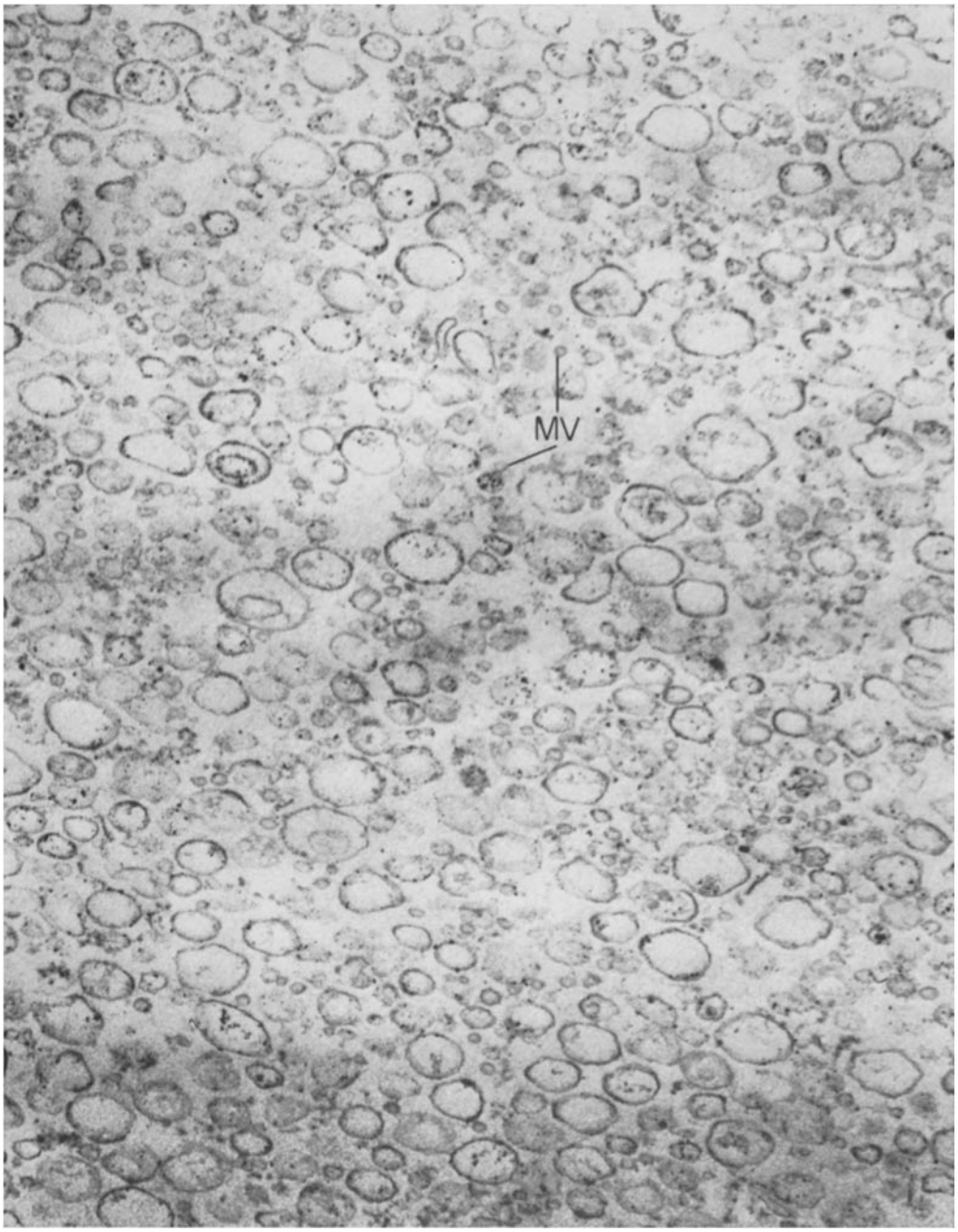

FIGURE 4 Electron micrograph of microsomal pellet isolated by high speed centrifugation of rat cerebral cortex homogenate in water. Micrograph represents middle layer of a pellet obtained by the "slow" procedure. MV, microvesicles. × 45,000.

in sucrose was much more contaminated with lysosomes than the microsomal pellet isolated in water. The relative specific activity of acid phosphatase (percentage of total activity/percentage of total protein) contaminating the microsomal pellet isolated in water was 0.8 as compared with 1.5 for the acid phosphatase of the pellet isolated in sucrose.

TABLE I

Influence of the Fractionation Medium on the Intracellular Distribution of Cerebral Enzymes and Proteins

Enzyme	Centrifugation medium	Subcellular fraction		
		NML	Microsomes	Soluble
		%	%	%
Cytochrome oxidase	Water	97.4	2.3	0.3
	Sucrose (0.25 M)	95.5	4.5	0
Acid phosphatase	Water	44.5	9.7	45.8
	Sucrose	59.5	29.1	11.4
Esterase	Water	75.4	17.4	7.2
	Sucrose	49.1	32.3	18.6
Glutamine synthetase	Water	21.2	61.7	17.1
	Sucrose	19.3	27.1	53.6
Lactic dehydrogenase	Water	35.2	2.5	62.3
	Sucrose	32.6	8.4	59.0
Protein	Water	61.0	9.2	29.8
	Sucrose	56.0	19.6	24.4

NML is particulate fraction comprising nuclei, mitochondria, and lysosomes. Values are reported as percentage of the sum of the units per gram (for enzymes) or milligrams per gram (for protein) recovered in all the fractions.

Esterase, which represented the microsomal marker enzyme, was also distributed quite differently in the two media. In water fractionation, a large portion of esterase activity appeared in fraction NML and comparatively little was recovered in fraction S. The reverse was true in the sucrose fractionation. Although more of the total activity was recovered in the microsomal pellet isolated in sucrose than in the one isolated in water, the relative specific activities of esterase in either pellet remained comparable.

The soluble marker enzyme, lactic dehydrogenase, was found to be distributed similarly in both centrifugation media. As noted previously (14), glutamine synthetase retained its association with the microsomal membranes to a much greater degree when pellets were isolated in water than when they were isolated in sucrose. The relative specific activity of this enzyme in the pellet isolated in water was 5.4 as compared with 1.4 in the pellet isolated in sucrose. The eluting effect of sucrose was further evidenced by a corresponding increase in the relative specific activity of glutamine synthetase recovered in the high speed supernatant fraction isolated in sucrose.

To obtain further information on the enzymic composition of the microsomal pellet obtained by fractionation in water, the pellet was tested for the presence of two additional enzymes, mitochondrial aspartic transaminase (23) and microsomal ATPase (26). The response of these, as well as of the enzymes described above, to the treatment of the microsomal pellet with 15 mM and 150 mM NaCl, respectively, was examined. The pellet (fraction P) was resuspended in the two concentrations of NaCl by manual homogenization. This suspension is referred to as fraction PS_1. Centrifugation at 145,000 *g* for 60 minutes yielded a pellet (fraction P_1) and a supernatant (fraction S_1). The effect of the salt treatment on the residual enzymic makeup of fraction P_1 is shown in Table II. Cytochrome oxidase remained bound to particulate matter, in contrast to aspartic transaminase, which was appreciably eluted by 15 mM NaCl and more so by 150 mM NaCl. It should be emphasized, however, that the activities of these mitochondrial enzymes in fraction PS_1 represented only a small fraction (< 3 per cent) of the total activity present in the unfractionated homogenate. Acid phosphatase was not eluted to a significant extent (< 13 per cent) by treatment with either concentration of salt. Lactic dehydrogenase, on the other hand, was almost completely solubilized by either salt concentration. The microsomal enzymes esterase and ATPase exhibited a comparable response to 15 mM and 150 mM NaCl. In either case, virtually all of the enzyme activity remained particulate. Microsomal glutamine synthetase partitioned differently between the particulate and the soluble phase depending upon

TABLE II

Partition of Cerebral Enzymes and Protein between Particulate and Soluble Fractions after Resuspension of the Microsomal Pellet Isolated in Water in 15 mM and 150 mM NaCl and recentrifugation

Enzyme	NaCl	Fraction			Percentage in P_1 ($P_1 + S_1 = 100$)
		PS_1	P_1	S_1	
	mM				
Cytochrome oxidase	15	7.7	8.8	0	100
	150	7.7	8.1	1.3	85
Aspartic transaminase	15	309	158	150	51
	150	309	78.5	190	35
Acid phosphatase	15	35.2	25.8	3.9	87
	150	35.2	20.7	2.6	89
Glutamine synthetase	15	35.0	31.6	2.3	93.2
	150	35.0	9.6	25.4	27.5
Esterase	15	17.4	15.6	1.50	91
	150	17.4	14.2	1.72	89
ATPase	15	13.7	10.2	0.6	94
	150	13.7	8.9	0.5	95
Lactic dehydrogenase	15	538	48	460	9.4
	150	538	66	602	10.0
Protein	15	4.6	3.5	0.7	83
	150	4.6	3.2	1.2	73

For definition of fractions, see Methods.
Enzyme activities are expressed in absolute values (units per gram) (see Methods); protein is expressed in milligrams per gram.

whether the low (retaining) or the high (eluting) concentration of NaCl was used. Thus, 72.5 per cent of the particulate enzyme activity was solubilized by 150 mM NaCl as compared with 6.8 per cent solubilized after treatment with 15 mM NaCl.

In addition to protein, the RNA, phospholipid phosphorus, and ganglioside *N*-acetyl neuraminic acid contents of the microsomal pellets isolated in water were determined (Table III). The pellets isolated in water were found to contain about 9 per cent of the total tissue protein as compared with about 19.5 per cent found in the pellets isolated in sucrose. An average value of 580 μg RNA per gram was found for the pellet isolated in water, giving a (RNA)/(protein + RNA) ratio of 0.11. In the pellet isolated in sucrose, this ratio was found to be 0.03.

TABLE III

Composition of Cerebral Microsomal Pellet Isolated in Water

Component	Amount
RNA	580 μg/gm
Gangliosides, as *N*-acetyl neuraminic acid	1.39 μmoles/gm
Phospholipid, as phospholipid phosphorus	30.0 μmoles/gm
Protein	4.6 mg/gm

DISCUSSION

The use of sucrose for the isolation of microsomal fractions has been predicated on the assumption that the preservation of morphological and chemical integrity of microsomal structure is dependent upon a milieu of relatively high osmotic strength, by analogy with requirements for the preservation of mitochondrial and lysosomal structure.

The present results indicate that it is possible to maintain the morphological integrity of the microsomal fraction obtained from rat cerebral cortex using water as the isolation medium. Furthermore, chemical and enzymic analysis of this pellet gives results which indicate that microsomes obtained by fractionation in water are less contaminated by extramicrosomal enzymes than are those obtained using media of high osmotic strength, and are closer in chemical composition

to relatively pure microsomal fractions obtained by density gradient experiments. These results, therefore, tend to invalidate and, indeed, militate against the use of media of high osmotic strength for the isolation of cerebral microsomal fractions. The chemical and enzymic findings are strengthened by the structural appearance of the sectioned microsomal pellets under the electron microscope. Vesicular membranous structures are present in great abundance, in marked contrast to the crenated, irregularly shaped structures which characterize the microsomal fractions isolated in sucrose. The latter, however, are comparable in appearance to sucrose preparations examined by de Robertis *et al.* (5), Aghajanian (34), and Gray and Whittaker (35). The preponderant structures seen in the preparations obtained in water are vesicles about 100 to 200 mμ in diameter, as well as smaller, round vesicles which possess a distinct lumen and whose diameter ranges between 20 and 50 mμ. Vesicles with granules attached to their surface are seen only rarely. This is in agreement with the findings in electron micrographs of sections of cerebral cortical tissue obtained in this laboratory and in others (36): relatively few areas replete with rough endoplasmic reticulum could be visualized. The dimensions and appearance of the small vesicles, which are particularly numerous in microsomal pellets obtained by means of the "fast" centrifugation procedure (Fig. 3), suggest their identity with synaptic vesicles. de Robertis *et al.* (15), Whittaker (17), and Johnson and Whittaker (37) have recently studied the effect of hypotonicity on preparations of isolated nerve ending particles and have found that the synaptic vesicles contained within the nerve ending particles are resistant to rupture even after particles have been exposed for 1 hour to 0.06 M sucrose. This hypotonic treatment did result, however, in the total rupture of the nerve ending particles themselves. Utilization of these observations has led to the obtaining of preparations of synaptic vesicles characterized by a high specific activity of acetylcholine and a low content of lactic dehydrogenase. The microsomal pellet obtained by direct centrifugation of cerebral tissue in water (Table I) contains only 2.5 per cent of the total lactic dehydrogenase activity, more than 90 per cent of which could be removed by one saline wash (Table II). This enzymic evidence for the virtual absence of nerve ending particles, the cytoplasm of which is known to contain entrapped soluble lactic dehydrogenase (37), is substantiated by the electron micrographic evidence, since no intact nerve ending particles could be seen in these preparations (Figs. 3 and 4). The larger percentage of the total lactic dehydrogenase found in the microsomal pellet when centrifugation was carried out in sucrose (8.4 per cent) instead of water (2.5 per cent) (Table I) reflects a greater contamination of the pellet by intact nerve ending particles. Correspondingly, intact nerve ending particles may be readily discerned in micrographs of pellets isolated in sucrose (Figs. 1 and 2). These results thus confirm the disrupting effect of water on the membrane enveloping the nerve ending particle and the relatively greater resistance of the membrane enveloping the synaptic vesicle to the action of media of low osmotic strength.

The analysis of microsomal pellets isolated in water for mitochondrial marker enzymes reveals a relatively low degree of contamination. It is noteworthy that the relative contaminations of the microsomal pellet by cytochrome oxidase (2.3 per cent) (Table I) and aspartic transaminase (2.4 per cent) (Table II) are almost identical. The somewhat higher figures for the pellet isolated in sucrose can perhaps be attributed to those mitochondria which became entrapped within the nerve ending particles during homogenization by virtue of the "pinching off" of the nerve endings, a process instrumental in the genesis of the nerve ending particles.

The differences in distribution of lysosomal enzymes with relation to the isolation medium employed are demonstrated by the finding that 9.7 per cent of the total activity of acid phosphatase is recovered in the microsomal pellet isolated in water as compared with three times that amount (29.1 per cent) recovered in the corresponding fraction isolated in sucrose (Table I). Also, the levels of acid phosphatase in the soluble supernatant are about four times higher in preparations isolated in water, owing to the osmotic instability of cerebral lysosomes (38). In this laboratory the studies in which fractions containing lysosomes were subjected to gradations of hypotonicity have revealed a marked osmotic sensitivity of lysosomes (39). In this connection, a further complicating factor has been the observation that the osmotic response of cerebral lysosomes is dependent on the composition of the fraction undergoing the hypo-osmotic treatment. Thus, in unfractionated homogenates lysosomes are "stabilized" against rupture (F. de Balbian Verster,

unpublished observations), whereas in "light" mitochondrial fractions "stabilization" of the lysosomes against osmotic lysis may be achieved by the addition to the hypotonic medium of purified dog or beef brain gangliosides (39).

The relatively high percentage of the total esterase activity recovered in fraction NML isolated in water is difficult to interpret at this time. In spite of the apparent accumulation of esterase in this fraction, which actually occurred irrespective of the centrifugation medium employed, the relative specific activity of this enzyme is nevertheless highest in the microsomal pellets.

There were marked differences in the distribution of glutamine synthetase, and these were strictly dependent on the centrifugation medium. Moreover, it should be noted that, since the relative specific activity of glutamine synthetase in the water-isolated fraction P was about four times that of the enzyme in the sucrose-isolated fraction P, the substitution of water for sucrose resulted in a fourfold purification of the enzyme.

The RNA content of the microsomal pellets isolated by centrifugation in water (Table III) was intermediate between the values reported by Toschi (7) and those reported more recently by Wherrett and McIlwain (9). The calculated ratio (RNA)/(protein + RNA) of 0.11 was considerably higher than the ratio of 0.03 obtained for pellets isolated in sucrose (14). A value of 0.12 was calculated for the ratio RNA/protein, in agreement with values reported by Ernster *et al.* (40) and Dallner *et al.* (41) for this ratio in subfractions isolated by treatment of rat liver microsomes with deoxycholate (40) or by layering techniques (41), but it is higher by about 350 per cent than the corresponding ratio for guinea pig cerebral microsomes isolated in sucrose (9). Finally, the pellets isolated in water were found to contain about 30 μmoles of phospholipid phosphorus per gram equivalent of tissue, an amount approximately twice that reported for guinea pig cerebral microsomes (9). The values for ganglioside *N*-acetyl neuraminic acid)1.39 μmoles/gm), on the other hand, compared favorably with the values reported for guinea pig cerebral preparations (9) isolated in sucrose.

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