Electron Microscopy of Two Subcellular Fractions Isolated from Cerebral Cortex Homogenate. By Evelyn Petrushka* and Antonio Giuditta.‡ (*From the Departments of Pathology and Biochemistry, Albert Einstein College of Medicine, Yeshiva University, New York.*) §

A considerable amount of information on tissue particulates has been obtained from combined biochemical and morphological investigations carried out mainly with liver and, to a lesser extent, with pancreas and muscle. There have been numerous biochemical studies with "mitochondrial fractions" isolated from 0.25 M sucrose homogenates of nervous tissue involving the oxidation of citric acid cycle intermediates (1–6), glycolysis (2, 4, 7), and oxidative phosphorylation (1, 3, 8–12). The criteria described by some of these authors (1, 12) for determining the purity of the fractions and the degree of preservation of the particulates are not completely satisfactory, being based on the low resolution of light or phase contrast microscopy.

A recent publication from this laboratory described a study of DPNH[1] oxidation in rat cerebral cortex (13). To define in greater detail the intracellular distribution of the enzyme systems catalyzing this reaction it was found convenient to fractionate the 0.88 M sucrose homogenate into seven separate fractions. It was found that the pattern of distribution of the antimycin A sensitive pathway of DPNH oxidation corresponded to that accepted for enzymes held to be associated with mitochondria in other tissues, namely succinate–cytochrome *c* reductase and cytochrome *c* oxidase. On the other hand, the distribution of the antimycin A insensitive pathway of DPNH oxidation was found somewhat similar to that of enzymes which have been considered to be associated with microsomal fractions; *i.e.*, esterase and 5′-AMPase. Since the patterns found were roughly similar to those observed with liver (14) and since previous workers have reported on biochemical properties of subcellular fractions isolated from brain (1–12), it was of interest to examine the morphological appearance of these fractions. Fractions 3 and 7 were selected for initial morphological study because, as in the case of liver (14), they possessed enzymic properties generally attributed to mitochondria and microsomes respectively. Phase contrast examination of samples of these fractions gave inconclusive results and it was therefore decided to study them by electron microscopy. This note describes the electron microscopic study.

* Aided by a Postdoctoral Fellowship from the American Cancer Society, Inc.

‡ Postdoctoral Fellow of the National Multiple Sclerosis Society.

§ *Received for publication, March 2, 1959.*

[1] Abbreviations used are as follows: DPNH = reduced diphosphopyridine nucleotide; 5′-AMPase = 5′-adenosine monophosphatase; TPNH = reduced triphosphopyridine nucleotide; RNP = ribonucleoprotein.

Methods

Rat cerebral cortex was obtained by slicing with a sharp blade, keeping to a minimum the amount of contamination with white matter. The 0.88 M sucrose homogenate therefrom obtained by using the all glass Dounce homogenizer, was separated into seven successive fractions by differential centrifugation. Fractions 1 to 6 were obtained by centrifuging for 10 minutes at 1000, 3200, 9500, 26,360, 64,590, and 105,400 *g* respectively and fraction 7 for 120 minutes at 105,400 *g* (13, 14). It was found advisable not to wash the fractions in order to keep to a minimum the possible biochemical changes resulting from this procedure. Representative portions of the sediments of fractions 3 and 7 were immersed in cold 1 per cent OsO_4-0.88 M sucrose solution, cut into small fragments at right angles to the surface of the pellet (*i.e.*, in the direction of the centrifugal force) and kept in the fixative for 1 hour at 2–4°C. The fragments were rinsed in cold Na-veronal-acetate buffer, pH 7.2, and kept overnight at 2–4°C. in 1.4 per cent formalin in veronal buffer (15, 16). Following rapid dehydration in increasing concentrations of ethanol, the pieces were suspended and embedded in butyl-methyl methacrylate containing uranyl nitrate according to the method of Ward (17). Polymerization was carried out in an oven at 45°C., overnight. Thin sections were cut by the Porter-Blum microtome and examined in an RCA-EMU 3B electron microscope.

Small pieces of rat-brain cortex, used for another purpose requiring preincubation in isotonic sucrose for 10 minutes at 22°C., were fixed in 1 per cent OsO_4-0.25 M sucrose for 15 minutes at 0°C. and processed for electron microscopy in identical fashion.

RESULTS

Sections cut at different depths of the fixed fragments exhibited no essential differences in the distribution of the various particle types.

Fraction 3:

A relatively low magnification micrograph of a representative field of fraction 3 (which in liver is largely mitochondrial (14)) reveals the high degree of heterogeneity in the population of particles sedimented at this gravitational force (Fig. 2).

There are many mitochondria which vary in shape, size, and state of preservation. They are readily identified by their internal cristae (18) and external membranes (Figs. 2, 3, 5). Some mitochondria show considerable deformation (*cf.* Novikoff, reference 19), or appear swollen when compared to mitochondria fixed "*in situ*" (Fig. 1).

The large concentrically laminated bodies are presumably myelin. They appear to be more or less distorted and possibly in various stages of fragmentation (Figs. 2, 3).

A fairly large quantity of membranous material is found in this fraction. This may be microsomal (Fig. 2); if so, this observation has its counterpart in the relatively high proportion of so called "microsomal" enzymes; *i.e.*, esterase, 5′-AMPase, TPNH-cytochrome *c* reductase, and antimycin A insensitive DPNH oxidase (13). The membranous material does not consist of granule-studded profiles or of vesicles bearing granules on the outer surface, as might be expected from comparable liver fractions (20). Rather, single membranes are present which follow a winding and irregular pattern with only occasional granules attached to them. It might be tentatively inferred that this particular pattern may have occurred as a response by the ergastoplasm of cerebral cortex cells to the procedures of homogenization and centrifugation in which membranes (endoplasmic reticulum) and granules (presumably RNP-rich) (20) become separated. Alternatively, these membranes may have been derived from the Golgi apparatus or the "agranular reticulum" of Palay and Palade (21) or from other membranes without granules such as myelin. Further work is required for the firmer cytologic identification of the membranes.

Also present in this fraction are round or oval electron opaque bodies (Fig. 2), which may be similar to particles described in whole neurons (22, 23).

Fraction 7:

Fraction 7, which in liver is essentially microsomal (14), is also heterogeneous (Figs. 4, 6, 7). Only an occasional mitochondrion is present. This is consistent with the biochemical data which show no appreciable enzyme activities associated with mitochondria; *i.e.*, cytochrome *c* oxidase and succinate–cytochrome *c* reductase. Few structures are seen which are readily recognizable as membranes belonging to or having been derived from the endoplasmic reticulum such as those described in fraction 3. A correlation of "microsomal" enzymes with membranes in brain fractions must await further work.

In this fraction (Figs. 6, 7) and to a lesser extent in fraction 3 (Fig. 2) granules may be seen which appear not to be attached to any membrane but seem to occur in clusters or aggregates. These are comparable to the free (presumably RNP-rich) granules found in nerve cells (21, 22); although unlikely, the granules may have been detached from the membranes of the endoplasmic reticulum. The small round electron opaque bodies described in fraction 3 are more numerous in this fraction (Figs. 6, 7). It is not unlikely that at least some of these images are produced by sections passing through the edge of a mitochondrion (*e.g.* in Fig. 2) or of an elongate electron opaque structure described below, yielding an apparently small ovoid particle.

The most prominent feature of fraction 7 is the presence of two structures which we are unable to identify but which, for the purpose of labelling, are referred to as *x* and *y*. One type, *x*, is represented by elongate electron opaque bodies of variable size, shape, and density which show no evidence of an enclosing membrane (Figs. 4, 7). The other type, *y*, is considerably less electron opaque, appears as a ribbon-like structure with somewhat darker rims and assumes various shapes but generally forms spheres of various sizes (Figs. 4, 6, 7). It remains to be established if all these appearances reflect the plane of sectioning of one type of body.

DISCUSSION

It is clear from this study that fraction 3 which has a high content of "mitochondrial" enzymes and fraction 7 which is essentially "microsomal" are both highly heterogeneous when examined with the electron microscope. Although these results were hardly unexpected in view of the known variety of cell types present in cerebral cortex and of the complexity of the individual cells, these electron microscopic studies of isolated cell fractions are of significance, particularly in the light of the growing number of biochemical studies made with such preparations.

Some of the particles in the fractions cannot be identified at present; nor can they be readily correlated with structures known to be present in tissues like liver or pancreas which have been more extensively studied.

It would, at present, be hazardous to assign a given enzymic activity to morphological entities of nervous tissue homogenates on the basis of analogy

with other organs. The data here obtained point to the difficulties involved in applying to brain and nervous tissue in general the separation techniques developed for another tissue like liver and to the need for modifications in the standard methods of separation. The judicious application of electron microscopy to these and similar studies should eventually enable investigators to distinguish the components and define the cytological and functional characteristics of relatively "pure" fractions isolated from nervous tissue cells.

We are grateful to Dr. Alex B. Novikoff and Dr. Harold J. Strecker for their guidance and criticism throughout the course of this work and in the preparation of the manuscript.

We wish to thank Mr. L. Jay Walker for the photographic work.

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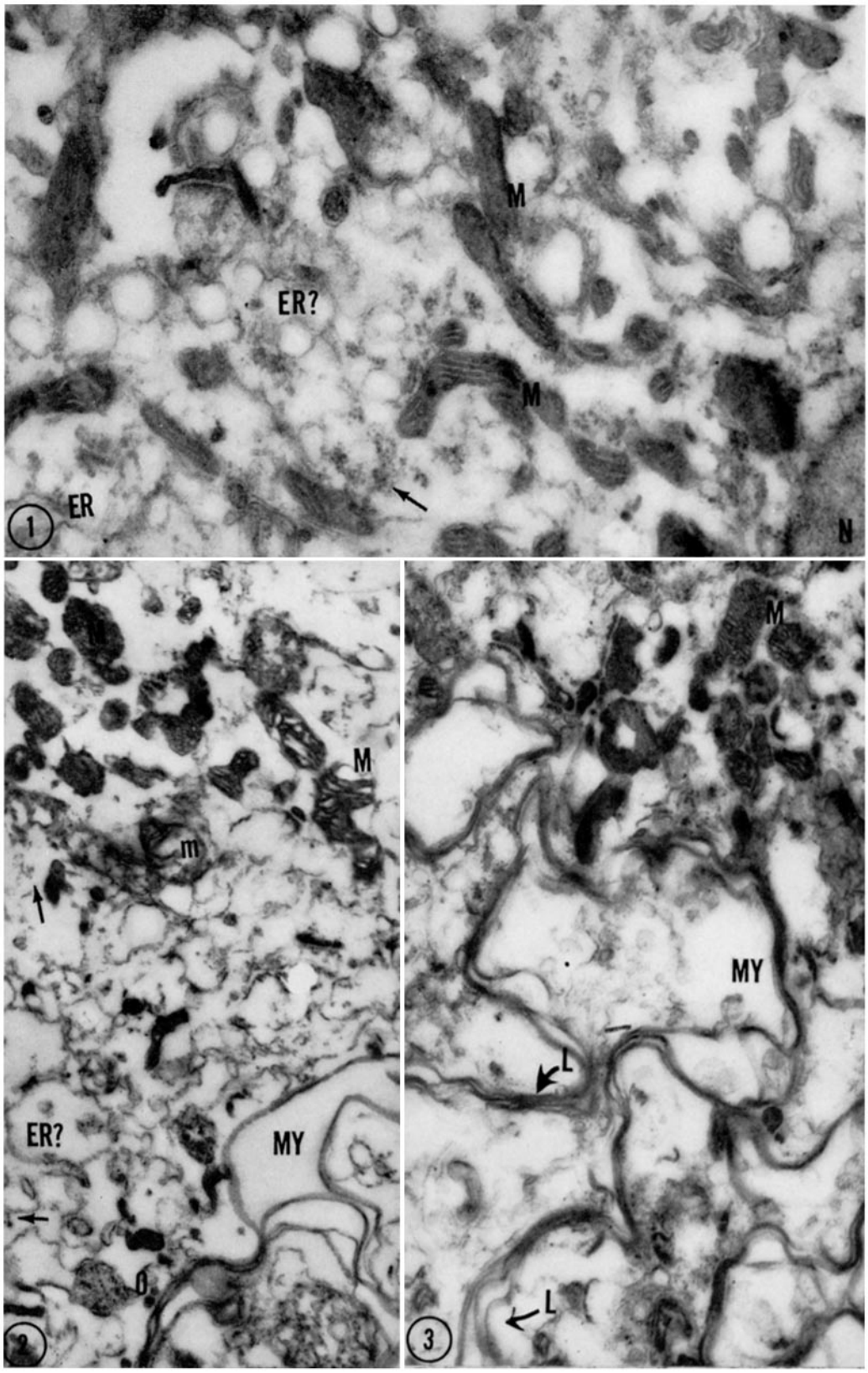
Explanation of Plates

Plate 82

Fig. 1. Portion of a cell from the cerebral cortex of the rat. It shows part of the nucleus (*N*), mitochondria (*M*) of different sizes and shapes, endoplasmic reticulum (*ER*), cytoplasmic vesicles which probably form part of the endoplasmic reticulum (*ER*?) and RNP granules (see arrow). × 35,000.

Fig. 2. Section through a pellet of fraction 3. Note mitochondria with distinct but altered *cristae* (*M*), damaged mitochondria (*m*), membranous forms probably derived from the endoplasmic reticulum (*ER*?), some RNP granules (see arrow), myelin (*MY*) and small electron opaque bodies (*O*). × 20,500.

Fig. 3. Section through another level of the same pellet of fraction 3. Note mitochondria (*M*) and myelin (*MY*) with characteristic laminations (*L*). × 20,500.



(Petrushka and Giuditta: Cerebral cortex homogenate)

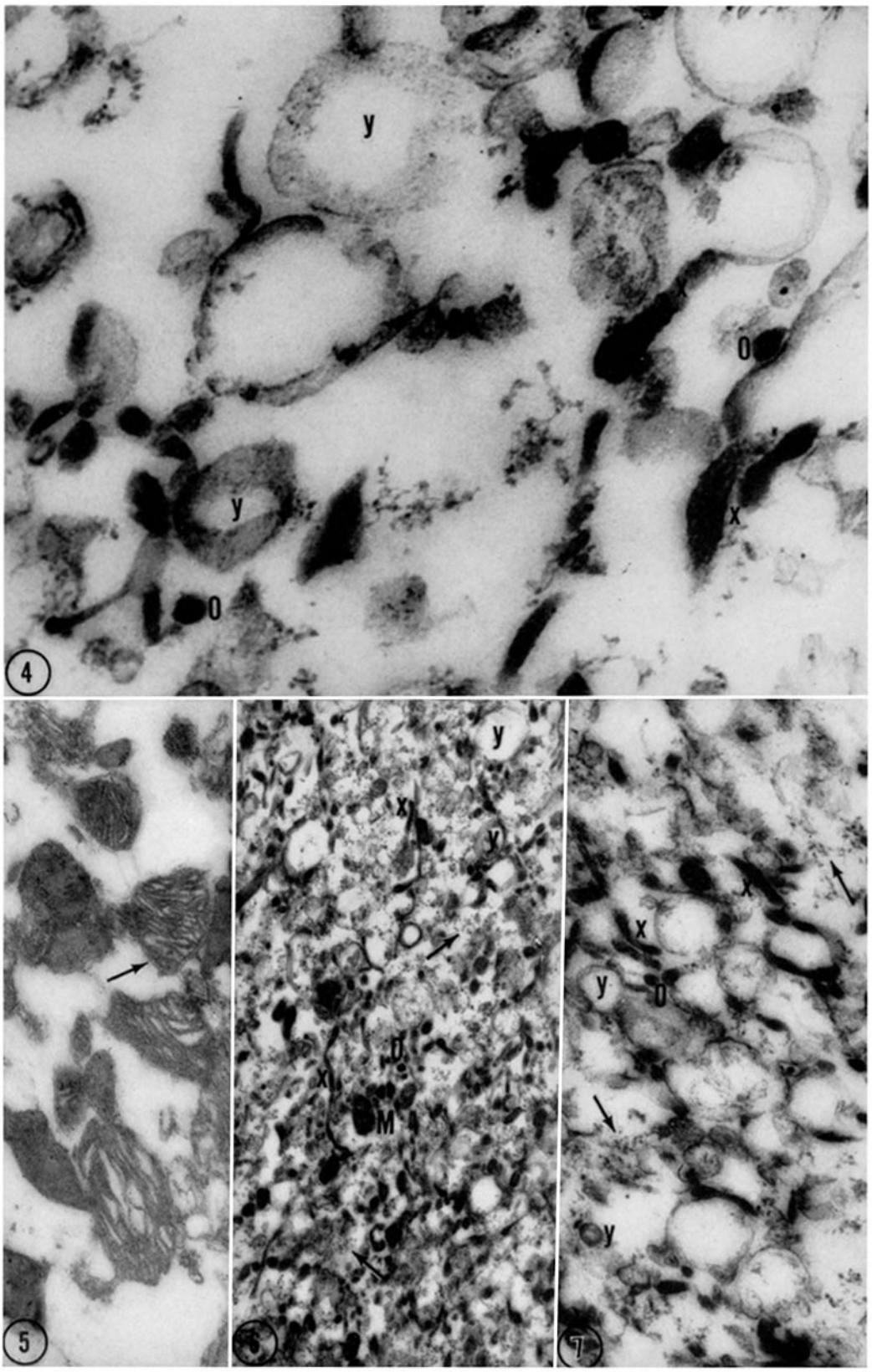
PLATE 83

FIG. 4. Higher magnification of section from fraction 7 showing details of unidentified structures, designated *x* and *y*. Note variability in size, shape, and electron opacity of both structures. Note the apparent absence of enclosing membranes about the very opaque, elongated particle *x* and the defined margins of the less opaque ribbon-like particle *y*. Also present are electron opaque ovoid bodies (*O*). × 59,000.

FIG. 5. Higher magnification micrograph of mitochondria from fraction 3 showing external membrane (arrow) and well defined but altered *cristae*. × 30,000.

FIG. 6. Section through a pellet of fraction 7, illustrating high degree of heterogeneity. Note a well preserved mitochondrion (*M*), clusters of RNP granules (see arrow), electron opaque bodies (*O*), and unidentified bodies (*x* and *y*). × 25,000.

FIG. 7. Section through a pellet of fraction 7 showing RNP granules (arrow), electron opaque bodies (*O*), and unidentified structures (*x* and *y*). × 35,000.



(Petrushka and Guiditta: Cerebral cortex homogenate)