# A RAPID METHOD OF OBTAINING SYNAPTOSOMES FROM THE RAT CEREBRAL CORTEX

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A rapid method of isolating differentiated synaptosomes from rat brain tissue is described. The electron-microscopic characteristics of the fractions, their distribution of activity of the enzymes monoamine oxidase, Na,K-adenosine-triphosphatase, inosinediphosphatase, and acid phosphatase, and their incorporation of precursors of protein synthesis and RNA in vivo are described.

The study of enriched fractions of nerve endings (synaptosomes) [8, 10] has shed new light on the biochemistry of synapses [1]. Before the functions of biologically active substances and the biosynthetic processes can be investigated in synaptosomes, a rapid method of their isolation is essential. This paper describes an attempt to develop such a rapid method of isolating differentiated synaptosomes.

Subcellular fractionation of the cerebral cortical tissue of rats weighing 110-120 g was carried out at 4°C, using the Beckman Spinco L-2 preparative ultracentrifuge. A 10% homogenate in 0.32 M sucrose with 0.05 M tris-HCl (pH 7.4) was centrifuged at 1000 g for 10 min. Since a lower yield of the fraction of "coarse" mitochondria (CM) was preferred to contamination by microsomes, the CM were isolated from the supernatant at 10,000 g (20 min) and then washed under the same conditions. The postmitochondrial fraction of the supernatant was centrifuged (55-60 min) at 18,000 g and "coarse" microsomes obtained in the residue. The CM were separated by centrifugation at 130,000 g in a stepwise sucrose density gradient by De Robertis's method [7] in the writers' modification. To obtain differentiated synaptosomes in a purer form, a gradient (No. 1) consisting of equal volumes of 0.8–1.0–1.1–1.2 M sucrose was used. The "purest" mitochrondria were obtained by the use of a gradient of 0.8–1.0–1.15–1.3 M sucrose, this time by centrifugation for 60 min. The gradients were carefully layered from cold sucrose solutions 30-60 min before fractionation. To obtain myelin in a purer form, the CM were suspended in 0.8 M sucrose (final concentration ~0.6 M). The following fractions were obtained: myelin (at the 0.6-0.8 M gradient boundary), synaptic membranes, light synaptosomes, heavy synaptosomes, and "pure" mitochondria (residue). The material of the fractions was diluted with 0.15 M sucrose and centrifuged at 20,000 g (40 min), and the residue was suspended in the corresponding incubation medium and kept at −20°C (if necessary). By the method suggested, differentiated synaptosomes can be isolated in 2.5-3 h. From 300 mg rat cerebral cortical tissue about 8 mg CM protein was isolated.

Electron-microscopic investigation of the fraction (Fig. 1; Table 1) showed a high level of enrichment and a satisfactory state of preservation of the structures. As was to be expected [8], monoamine oxidase (MAO) activity was maximal in the "pure" mitochondria, and transport adenosinetriphosphatase (ATPase) was maximal in the synaptic membranes (Table 2). The lysosomal enzyme, acid phosphatase, was diffusely

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TABLE 1. Electron-microscopic Characteristics of Subcellular Fractions

| Fractions | Characteristics |
|---|---|
| Myelin | Besides myelin fragments, myelinated axons (up to 15%) were present with their axoplasm intact (mitochondria and neurotubules visible); the mitochondria were virtually uncontaminated by synaptosomes |
| Synaptic membranes | Mainly "ghosts" of synaptosomes with washed-out cytoplasm or large membrane fragments; up to 20% of myelin, 15% of small synaptosomes, and 15% small membrane fragments |
| Light synaptosomes | Mainly synaptosomes with "light" cytoplasm, some with postsynaptic membranes; up to 20% of membrane material |
| Heavy synaptosomes | Mainly synaptosomes with "dense" cytoplasm, containing more mitochondria than the light fraction; up to 20-35% of mitochondria |
| "Pure" mitochondria | Mitochondria with intact cristae; up to 20-30% contamination by synaptosomes and membrane material |
| "Coarse" microscomes | Small fragments of disintegrated membrane structures of endoplasmic reticulum and plasma membranes; some membranes (20%) as "microsomal" vescicles, up to 10-20% contamination with synaptosomes |

Note. In all fractions dense osmiophilic structures, evidently lysosomes, are found. When gradient No. 1 was used, the fractions obtained in the most enriched form were myelin, synaptic membranes, and light and heavy synaptosomes, while when gradient No. 2 was used, the corresponding fractions were mitochondria, light synaptosomes, and myelin.

TABLE 2. Metabolic Characteristics of Subcellular Fractions

| Characteristics | Fractions | | | | | |
|---|---|---|---|---|---|---|
| | "coarse" microsomes | myelin | synaptic membranes | light synaptosomes | heavy synaptosomes | "pure" mitochondria |
| Protein content (% of CM) | — | 8 | 11 | 16 | 37 | 23 |
| Specific activity of protein synthesis | 32 992 ±2 855 | 14 657 ±2 697 | 13 901 ±159 | 15 942 ±794 | 18 735 ±653 | 19 879 ±1 438 |
| Specific activity of RNA synthesis | 6 639 ±866 | 2 134 ±97 | 3 040 ±525 | 1 122 ±72 | 972 ±21 | 2 446 ±245 |
| MAO activity | 10,4 ±1,5 | 0 | 0 | 15,0 ±2,1 | 21,0 ±2,2 | 59,7 ±8,7 |
| Activity of Na, K-ATPase | 8,3 ±1,1 | 9,7 ±0,1 | 27,4 ±1,7 | 19,8 ±1,4 | 9,6 ±1,0 | 5,6 ±0,7 |
| IDPase activity | 5,9 ±0,6 | — | 6,2 ±0,5 | 3,0 ±0,4 | 5,15 ±0,45 | 0 |
| Acid phosphatase activity | 3,94 ±0,4 | 5,2 ±0,3 | 3,47 ±0,1 | 2,93 ±0,07 | 3,52 ±0,4 | 2,73 ±0,15 |

Note. Specific activity in both cases given as pulses/min/mg protein [1, 5] after intracerebral injection of lysine-$H^3$ and orotic acid-$C^{14}$ 3 h before decapitation. MAO activity in $\mu$moles substrate (P-nitrophenylethylamine)/mg protein/min [4]. Activity of Na, K-ATPase calculated from difference between Na,K,Mg- and Mg-ATPase, expressed in $\mu$moles $P_{in}$/mg protein/h; activity of IDPase and acid phosphatase expressed in $\mu$moles substrate/mg protein/min [4]. Results given for gradient No. 1.

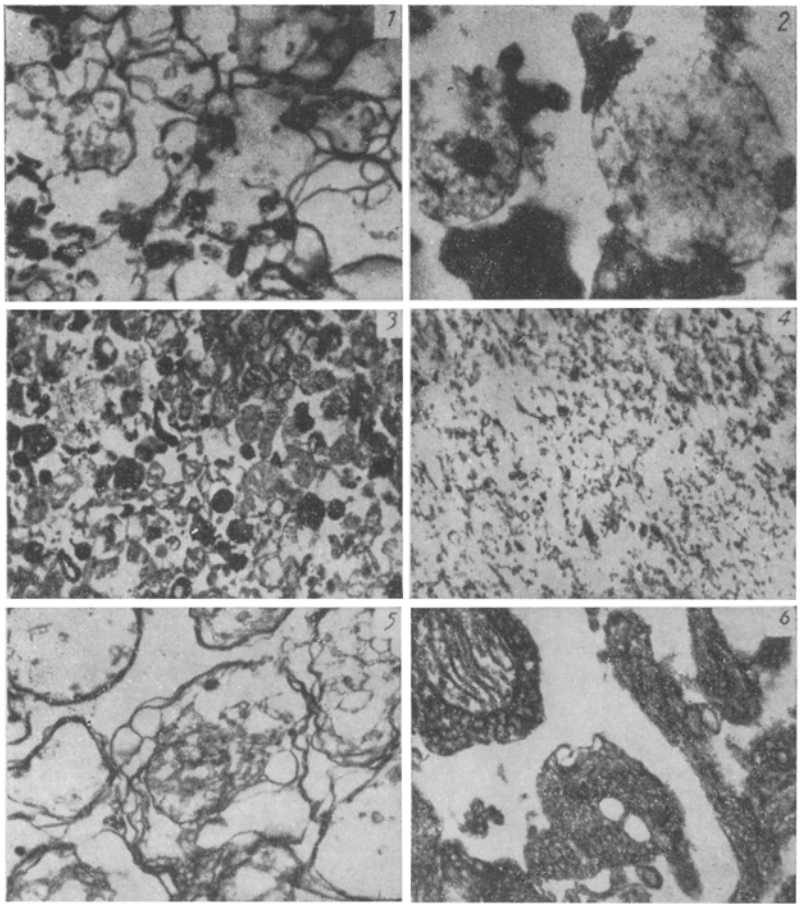

Fig. 1. Electron-microscopic characteristics of subcellular fractions: 1) synaptic membranes; 2) synaptic contacts, fragment of fraction CM; 3) "pure" mitochondria; 4) microsomes; 5) myelin; 6) heavy synaptosomes. Fixation in 0.5% $O_8O_4$ solution in 0.05 M tris-HCl (pH 7.4) containing 0.4 mole sucrose (40 min; 0-2°C). Embedded in Araldite, sections shadow-cast with uranyl acetate and lead citrate [2, 3].

distributed in the synaptic structures [10], and inosinediphosphatase (IDPase), regarded as marker of the microsomal membranes of the liver, was also found in these experiments in the synaptic membranes, although microsomal contamination in the synaptic structures, as shown by electron-microscopy and by the results of protein and RNA synthesis in vivo (Table 2), was minimal.

The rapid method described above has advantages over other methods [6, 9], for it enabled differentiated synaptosomes to be obtained and at the same time gives highly enriched fractions of myelin, light synaptosomes, and mitochondria.

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