sation. The smaller efflux increase in the 30 mM glucose medium corresponds to slower loss of *myo*-inositol from lenses of diabetic rats (Table I).

These observations show that in sugar cataracts the osmotic swelling due to hexitol accumulation leads to a reduced uptake and an increased efflux of *myo*-inositol, resulting in a low *myo*-inositol level in the lens.

When this work was concluded an abstract from the work of COTLIER[11] was published. His results are in agreement with our findings concerning the accumulation of *myo*-inositol by the normal lens and its inhibition by ouabain.

Capable technical assistance was rendered by Miss G. J. M. L. DE VRIES. The critical advice of Prof. Dr. S. L. BONTING is gratefully acknowledged.

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Received May 27th, 1968

Biochim. Biophys. Acta, 163 (1968) 269–272

BBA 73055

Isolation of a membrane fraction enriched in nerve-end membranes from rat brain by zonal centrifugation

Nerve-end particles (synaptosomes) from brain homogenates have been isolated by a combination of differential centrifugation and isopycnic banding[1–3]. Centrifugation of a crude mitochondrial fraction from rat brain in a titanium B-XV zonal centrifuge[4] yields two major bands, one of which has a prominent shoulder (isotonic sucrose profile, Fig. 1). The first zone (banding in 23 % sucrose (w/w)) consists of myelin and membrane fragments. Electron microscopy shows the second band at 35 % sucrose (w/w) to contain mostly nerve-end particles and a few free mitochondria. The shoulder at 38–41 % sucrose is a quite homogeneous population of free mitochondria.

Recently, nerve-end membranes (*e.g.*, plasma membranes originating from nerve endings) have been separated from an osmotically shocked crude mitochondrial fraction on discontinuous sucrose gradients[5]. When the crude brain mitochondrial fraction is subjected to osmotic shock and resolved on a linear sucrose gradient in a

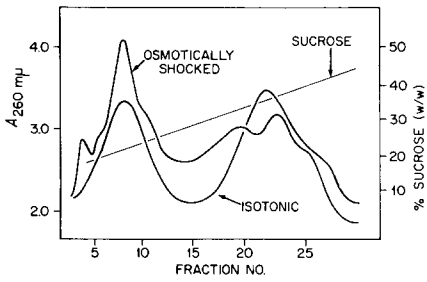

Fig. 1. Distribution of particulate material from an isotonically prepared crude mitochondrial fraction (lower trace) compared to an osmotically shocked fraction (upper trace). A 20% homogenate was prepared in ice-cold 10% sucrose (neutralized to pH 7 with NaOH) and diluted to 5% with ice-cold 10% sucrose for centrifugation. Nuclei and cell debris were removed by centrifugation at 3000 rev./min for 5 min in a rotor No. 30, and the crude mitochondrial fraction was isolated at 14 000 rev./min for 20 min. The fraction was either osmotically shocked or gently resuspended in 10% sucrose and applied to a sucrose gradient. The profile generated by particles treated only with isotonic 10% sucrose is shown in the lower trace (isotonic). Osmotic shock was carried out with cold distilled water (7 ml/g brain) for 20 min after which the sample was homogenized by hand. This profile is shown in the upper trace (osmotically shocked). The gradient was 15–50% sucrose (w/w) pH 7.0 (1200 ml with a 50% cushion) in a B-XV zonal rotor. The gradient was run for $5500 \cdot 10^7\ \omega^2 t$ (about 2 h at 27000 rev./min). At the completion of the run, the gradient was displaced with 55% sucrose and the absorbance light-scattering profile monitored by a flow-through cell in a DU spectrophotometer at 260 mμ. 40-ml samples were collected.

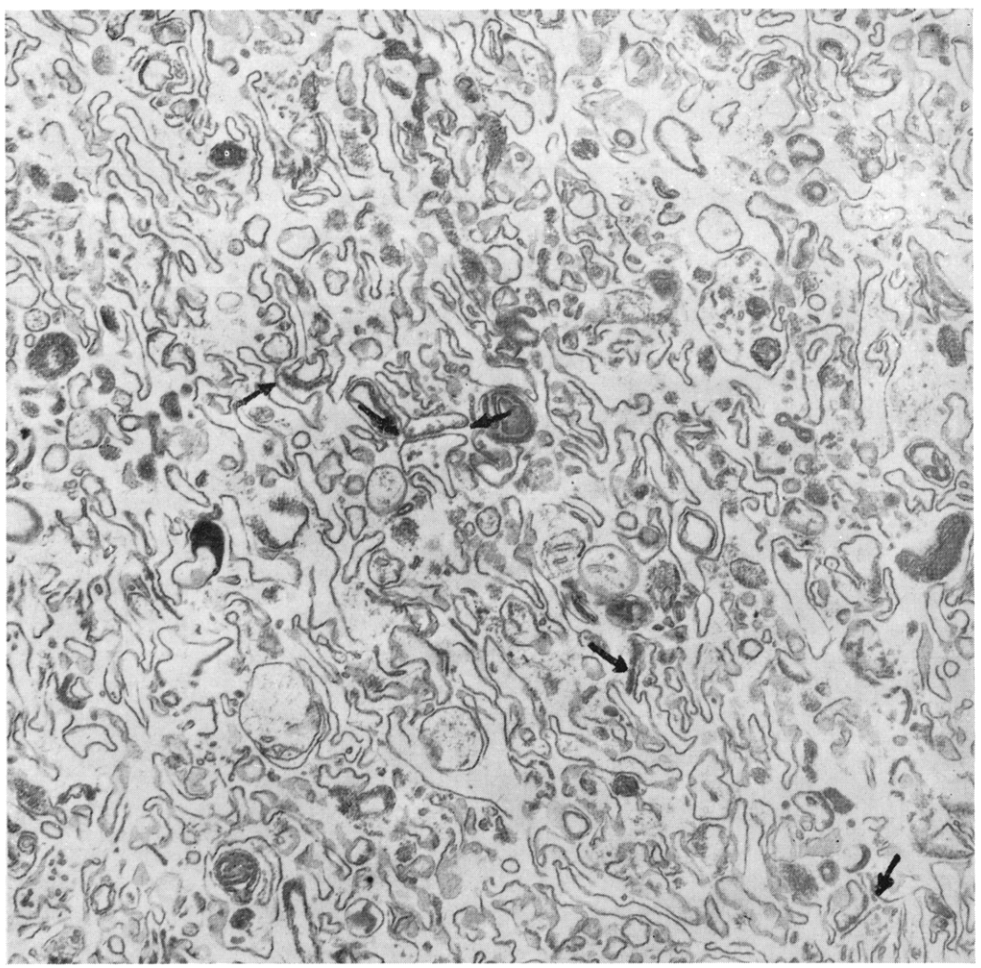

Fig. 2. Electron micrograph of a nerve-end membrane fraction collected (29.1–31.7% sucrose, w/w). Some synaptic thickenings can be seen (arrows). × 24000.

B-XV zonal rotor, an additional major particulate band is observed at the 32% (w/w) sucrose level (Fig. 1). Electron micrographs of this new zone reveals the band to consist almost entirely of free membranes. Many of these membranes show synaptic thickenings, permitting them to be identified as nerve-end membranes (Fig. 2). (Na^+-K^+)-activated ouabain-sensitive ATPase, a membrane marker enzyme contained in high specific activity in nerve-end membranes[5,6], is concentrated in this fraction. Enzymes characteristic of other subcellular particles are low. The specific activity of cytochrome oxidase, a mitochondrial inner-membrane enzyme[7], is low, as is that of antimycin-insensitive NADH-oxidase, a microsomal[8] and outer mitochondrial membrane enzyme[7] (Table I). Based on these enzyme analyses and particle counts with the electron microscope, the assayable contamination is in the neighborhood of 10% and is no higher than 20%.

TABLE I

COMPARISON OF ENZYME SPECIFIC ACTIVITIES IN VARIOUS FRACTIONS

ATPase was assayed by determining the difference between the amount of ATP hydrolyzed in the presence and absence of ouabain as described by HOSIE[6]. Cytochrome oxidase was measured by the procedure of DUNCAN AND MACKLER[10]. Antimycin-insensitive NADH oxidase was assayed by following the reaction of NADH at 350 mμ. The assay mixture contained 1 ml 0.05 M sodium phosphate, 0.20 mg of NADH, 0.20 mg of cytochrome *c*, 1 μg of antimycin A and enzyme. Data are averaged from 3 experiments.

Fraction	*(Na^+-K^+)-ATPase (units*/min per mg protein)*	*Cytochrome oxidase (units**/min per mg protein)*	*Antimycin-insensitive NADH oxidase (units***/min per mg protein)*
Homogenate, total	1.75 ± 0.5	1.2 ± 0.4	4.7 ± 0.8
Nerve-end membranes (from about 29–31% sucrose)	12.7 ± 1.7	0.6 ± 0.2	1.9 ± 0.6
Mitochondria (41% sucrose)		4.3 ± 1.2	11.4 ± 2.4
Microsomes			26.2 ± 7.1

* 1 unit = 1 μmole of inorganic phosphate released.
** 1 unit = 1 μmole cytochrome *c* oxidized.
*** 1 unit = μmoles of NADH reduced.

These results demonstrate that membranes released by osmotic shock from a nerve-end particle fraction can be collected as a discrete zone in regions of the sucrose gradient which are relatively free of particles before osmotic shock. It is seen that the consequences of osmotic shock are a diminished number of whole nerve-end particles and a new band showing the morphology and enzymatic characteristics of nerve-end membranes. Mitochondria and other membranes are unaffected by the osmotic shock; their density position remains relatively constant. Thus, the new band is collected at a density where other particles are not found and where contaminate levels are low. The membrane fraction is enriched in nerve-end membranes, but the contribution of plasma membrane fragments arising from neuron dendrites, soma, and possibly glial cells, not eliminated by the preliminary differential centrifugations, cannot as yet be evaluated. The density range established for the nerve-end-membrane band by the zonal method shows that RODRIGUEZ DE LORES ARNAIZ, ALBERICI AND DE ROBERTIS[5]

and other investigators[6] collect nerve-end membranes from the leading edge of this band.

Detailed studies on the use of combined rate and isopycnic zonal separation techniques[9] to the fractionation of brain will be presented in detail elsewhere.

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Received June 5th, 1968

Biochim. Biophys. Acta, 163 (1968) 272–275

BBA 73054

Spatial relationship between intestinal disaccharidases and the active transport system for sugars

With the present great increase in the number of proposals for the biochemical secret of membrane transport, it may be a truism, but nonetheless worthwhile to note (1) that there is a vast array of thermodynamically feasible mechanisms from which to choose and (2) the fact that one is feasible does not mean that it is actually used by the cell. Temporal or spatial juxtaposition of events as evidence for a mechanism are especially suspect and proposals based on such evidence should be subjected to rigorous tests at the earliest moment. The recent suggestion[1] that membrane-bound trehalase is the terminal catalyst in a reaction sequence leading to the membrane active transport of glucose and other sugars is one of this sort. This particular speculation was directed primarily to the proximal convoluted tubule of the kidney but

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** The Molecular Anatomy Program is sponsored by the National Cancer Institute, the National Institute of General Medical Sciences, the National Institute of Allergy and Infectious Diseases, and the U.S. Atomic Energy Commission. The Oak Ridge National Laboratory is operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.