Distribution Between Free and Membrane-Bound Ribosomes in Rat Brain

Rat brain ribosomes appear in electron micrographs either freely scattered in the cytoplasm of the cell, or closely attached to the membranes of the endoplasmic reticulum[1]. Upon tissue homogenization the membranes are fragmented into vesicles and isolated as the microsomal fraction[2]. As judged from the pictures, there seem to be more free ribosomes than membrane-bound ribosomes in rat brain[3–5], but to our knowledge there have been no attempts made to quantitate the distribution of these ribosomes.

In order to investigate this question, rats were injected intracerebrally[6] with 5 μC uridine-2-C^{14} (55.2 mC/mM) or 5 μC adenosine-8-C^{14} (28.4 mC/mM). After 3–14 days labeling the rats were decapitated and the brains were homogenized in 3.5 volume of buffer as described by BLOBEL and POTTER[7]. The post-mitochondrial supernatant (7 ml) was layered on top of a 6 ml linear sucrose gradient (from 2.2M to 0.25M). Following centrifugation for 16–18 h at 35,000 rev/min (150,000 g average) in a Spinco SW41 rotor, the bottom of the centrifuge tube was pierced and 40 fractions (15 drops through a 20 gauge needle) were collected. The contents of every other tube were precipitated with 5% trichloroacetic acid, filtered through nitrocellulose membrane filters and the radioactivity was determined in a liquid-scintillation counter[6].

The radioactivity profile (Figure 1) revealed two bands: the main peak at the bottom of the tube (due to free ribosomes), and a small area in the middle of the gradient (due to membrane-bound ribosomes). The identities of these bands were established by electron microscopic examinations of the high speed pellets from these areas (Figures 2 and 3). The pellets were fixed in glutaraldehyde, postfixed in osmic acid, embedded in Epon 812, sectioned and stained with uranyl acetate and lead hydroxide.

Further treatment of the membrane-bound ribosomes with a detergent (0.3% solution of Triton X-100) solubilized the membranous structures. When the resulting mixture was layered on top of a sucrose gradient and centrifuged once more in the SW41 rotor, the bulk of the radioactivity had moved to the bottom of the tube and little radioactive material was found in the middle of the sucrose gradient where originally the membrane-bound ribosomes were found. This indicates that the radioactivity was associated with heavy particles which, once liberated from the membranes by the detergent, sedimented like free ribosomes.

The question still remained whether the distribution of the radioactivity along the sucrose gradient could be used for quantitative identification of free and membrane-bound ribosomes. Since rat brain ribosomes contain 33% RNA[8], the specific radioactivities of the RNA's of the 2 radioactive bands in sucrose gradient were determined. In one set of experiments the RNA was extracted with 0.5M perchloric acid at 70 °C according to Schneider as described by CHEFTEL and BOUCHILLOUX[9]. In another set of experiments a second portion of the ribosomes was treated with Triton X-100, and pelleted by centrifuging in a Spinco 50T rotor at 100,000 g for 1 h. The RNA was then hydrolyzed at 37 °C in 0.3N KOH for 18 h and the neutralized nucleotides were passed over a Dowex-1X10 (formate) column. The specific radioactivities of the eluted

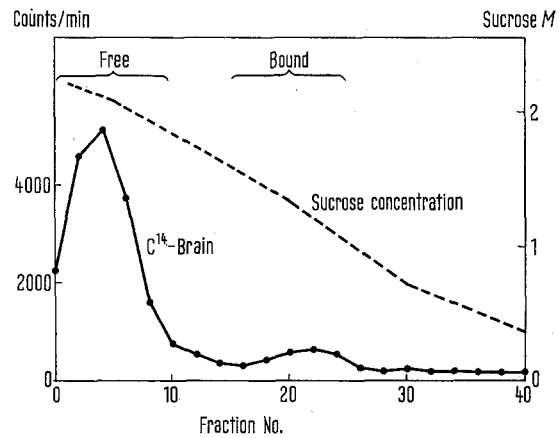

Fig. 1. Sedimentation pattern of rat brain ribosomes labeled with uridine-2-C^{14} for 3 days. Faster moving components are to the left.

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Comparison of specific radioactivities of free and membrane-bound rat brain ribosomes

Precursor injected	Ribosomes isolated	Specific radioactivities Counts/min / OD^{260} units				
		RNA extracted according to Schneider	Nucleotides isolated on Dowex-1 (Formate) column			
			CMP	AMP	GMP	UMP
Adenosine-C^{14}	free	1340	120	4400	2590	27
	membrane-bound	1460	115	2600	3780	55
Uridine-C^{14}	free	3040	14,910	440	190	9100
	membrane-bound	3150	10,020	248	46	6130

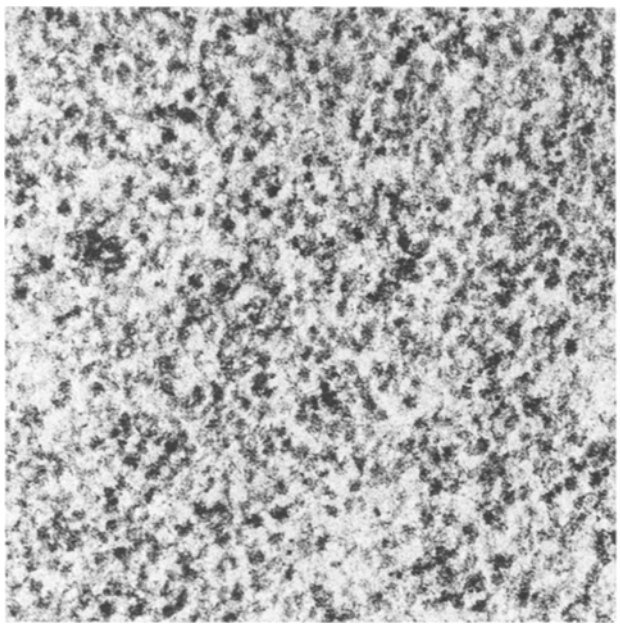

Fig. 2. Free ribosomes of rat brain. × 100,000.

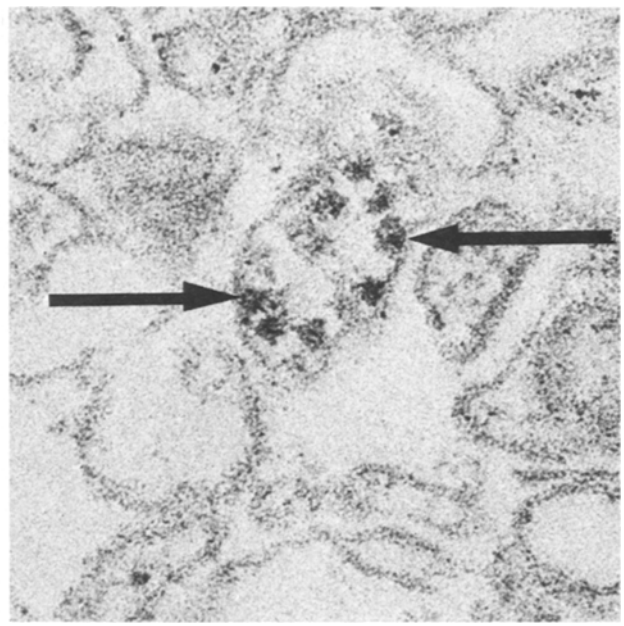

Fig. 3. Membrane-bound ribosomes (arrows) of rat brain. × 200,000.

nucleotides were determined as described by MERITS and CAIN[6].

The specific radioactivities of the RNA extracted from free and membrane-bound ribosomes were remarkably similar (Table) for both radioactive precursors used. When adenosine-8-C^{14} was used as precursor, the label of the ribosomal RNA appeared in both purine nucleotides. Likewise injection of uridine-2-C^{14} rendered both pyrimidine nucleotides radioactive. The specific radioactivities of the isolated nucleotides were somewhat erratic.

The continuous sucrose density gradient centrifugation method was used for 26 determinations of the distribution of brain ribosomes using a radioactive pulse from 3–14 days. An average of 14.2% of the total ribosomes were found to be membrane-bound (range 8.0–19.5%).

The separation of labeled ribosomes into 2 bands was further tested with rat liver microsomes. Rats were injected i.p. with 2 mC uridine-6-H^3 (9.34 C/mM) or 2 mC adenosine-H^3 (generally labeled, 2.34 C/mM). In 8 experiments with 3–7 days labeling time, an average of 69% of the total ribosomes were found to be membrane-bound (range 57–79%). These findings agree well with the results obtained by other workers[7].

Zusammenfassung. Der Anteil von membrangebundenen Ribosomen im Verhältnis zu den freien Ribosomen für Gehirngewebe wird festgelegt (14%), und die Gradientenbänder werden elektronenmikroskopisch kontrolliert.

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Electron Histochemical Evidence of Different Types of Mossy Fibre Endings in the Cerebellar Cortex

Until recently there has been no general agreement among physiologists, concerning cholinergic transmission in the cerebellar cortex. According to CURTIS and CRAWFORD[1] it is not acetylcholine (ACh) but one or more acidic amino acids, e.g. glutamic, that play the part of transmitter substance, in contrast to the view expressed by PHILLIS[2] that the transmitter may be ACh.

In 1956 HEBB[3] found that in the developing cerebellar cortex the cholinacetylase (ChA) activity shows an early peak, but later decreases. She suggested that this early peak is due to the development of cholinergic neurones, while the subsequent decrease in activity per gram is due to the later increase of non-cholinergic fibres, which come to make up the bulk of the adult cerebellum.

CSILLIK et al.[4] have shown by optical microscopic observations that the rat archicerebellar cortex shows acetylcholinesterase (AChE) activity in the early developing cerebellar stage. KASA[5] obtained similar results in the develeping cerebellar cortex in different mammals.

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