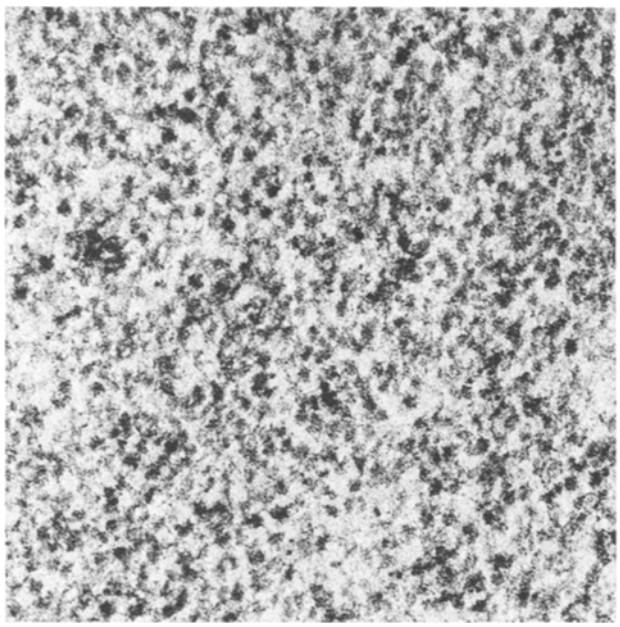

Fig. 2. Free ribosomes of rat brain. × 100,000.

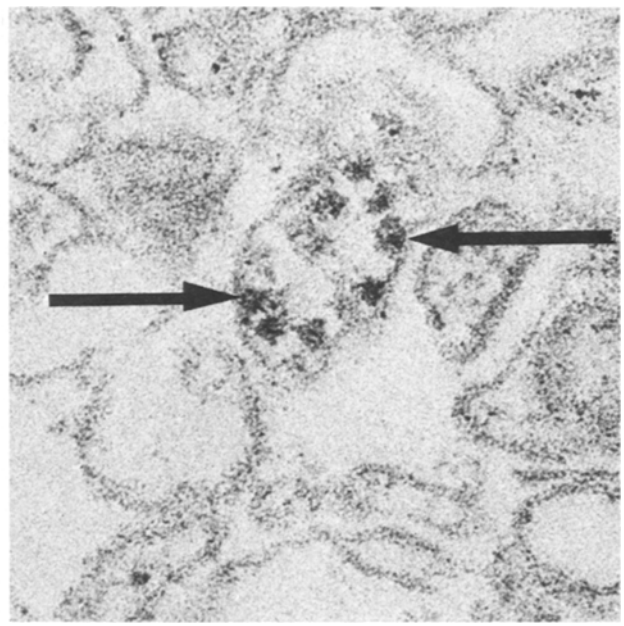

Fig. 3. Membrane-bound ribosomes (arrows) of rat brain. × 200,000.

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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[3] C. O. HEBB, J. Physiol. *133*, 566 (1956).
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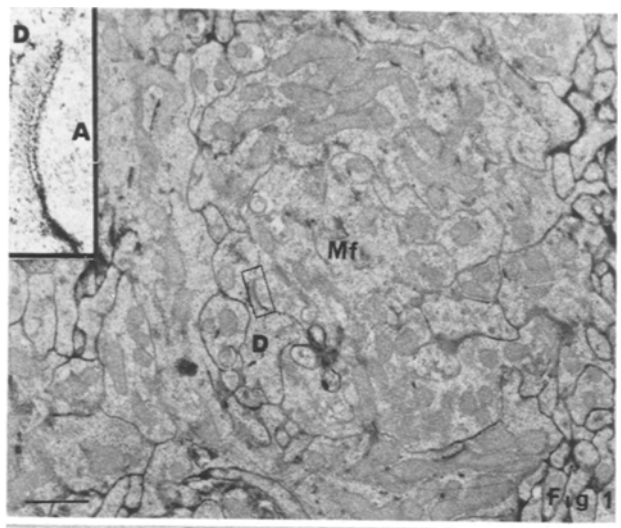
The presence of AChE in the mammalian cerebellar cortex has been well established by optical microscopic histochemical methods. Several investigators[6–8] (and many others) have shown that the enzyme is located in the cerebellar glomeruli, while others[9–11] have demonstrated its presence in the granular cell bodies (see SILVER[12]). At ultrastructural level, however, there have been a few reports[13,14] of mossy fibre activity. In the present electron histochemical studies, it was observed that there is a variation in AChE activity between the mossy fibres.

The work reported here was carried out on the cerebellar cortex of rat, rabbit, guinea-pig, cat and kitten. The samples were fixed in 4% formaldehyde and 2% glutaraldehyde mixture in 0.1 *N* sodium cacodylate buffer (pH: 7.4) before incubation. After fixation some of tissues were pretreated either with 10^{-6} *M* Mipafox or 10^{-4} *M* BW 284 C 51 solution for 30 min, thereafter the samples were incubated in copper-lead-thiocholine medium[15]. The end-product of the reaction was converted to the corresponding sulphides in 10% sucrose solution saturated with gaseous H_2S. The samples were postfixed first in 6% glutaraldehyde (3–5 min) then in 6% glutaraldehyde and 1% OsO_4 (5 min) and finally in 1% OsO_4. The tissues were dehydrated in ethanol and embedded in the usual way. Thin sections were cut on a Reichert ultramicrotome and examined in the JEM 6 C and Siemens electronmicroscopes.

The AChE of the mossy fibre terminals was found to vary in different mammals in the following manner: *Type I/a.* AChE activity appears on the entire surface of the mossy fibre endings (Figure 1). *Type I/b.* AChE reaction end-products can be seen only at some synaptic regions or other parts of the mossy fibre terminal. In both cases (a, b) the reaction end-product is present on the outer surface of the mossy fibre unit membrane, on the post-synaptic membrane and in between the 2 membrane surfaces. *Type II.* AChE activity is not present on the mossy fibre terminals although the other parts of the glomerulus exhibit enzyme activity (Figure 2). Of these types of terminals Type I/a occurs most frequently in the archicerebellar cortex, while Type II is characteristic of other parts of the cerebellar vermis.

It is reasonable to assume that differences in AChE activity may reflect differences in the nature of the chemical transmission. These electron histochemical investigations, together with other results[2,3], and with recent measurements of choline acetyltransferase in normal and isolated areas of different regions of the cerebellar cortex[16], support the idea[3] that at least some mossy fibres in the cerebellar cortex are cholinergic[17].



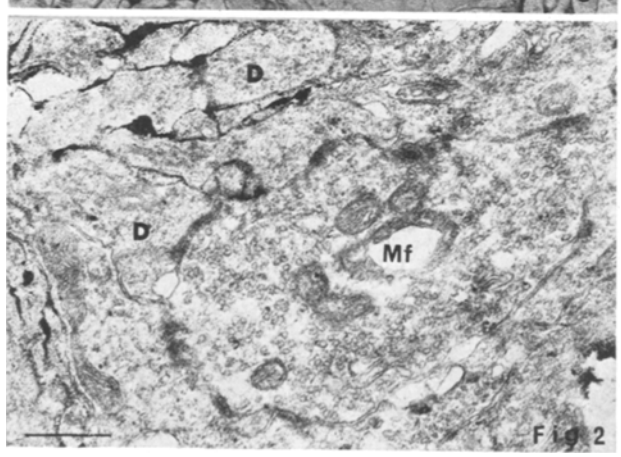


Fig. 1. AChE reaction end-product appears on the entire surface of the mossy fibre ending (Mf). At higher magnification (e.g. insett) it can be seen, this activity is on the axolemma (A) and granule cell dendrite membrane (D).

Fig. 2. AChE activity appears on the surfaces of granule and Golgi cell dendrite membrane but reaction end-product is not present on the mossy fibre terminal.

Zusammenfassung. Die Moosfasern endigen im Kleinhirn, nach elektronenmikroskopischen und histochemischen Untersuchungen, in Form von 2 Typen: der eine ist cholinergisch und wird zur Hauptsache im Archizerebellum gefunden, während der andere keine ACh-Aktivität zeigt und im Paläozerebellum verteilt ist.

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[17] The author wishes to express his thanks to The Wellcome Trust for supporting this work, Dr. C. O. HEBB, ARC Institute of Animal Physiology, Babraham, Cambridge (England) for the hospitality of her department and her interest, and to Dr. P. ROHLICH, Dept. of Histology, Budapest (Hungary) for the use of the JEM 6 C microscope.