Die cytologischen Daten von *M. sulcinodis* sind deutlich unterschieden von denen der anderen Vertreter der Gattung. Dieser Befund gewinnt an Interesse, da die taxonomische Unterteilung der Gattung *Myrmica* in die beiden Untergattungen *Neomyrma* und *Myrmica* von den cytologischen Befunden nicht unterstützt wird. *M. rubida*, die einzige Art der Untergattung *Neomyrma*, ähnelt cytologisch den Arten der Untergattung *Myrmica*, während *Myrmica* (*Myrmica*) *sulcinodis* von den Vertretern der eigenen Untergattung abweicht[5].

Summary. In the testes of *Myrmica sulcinodis* there are not only haploid and some diploid cells to be found, as in the testes of ***M. ruginodis, M. sabuleti*** and ***M. rubida,*** but a large mass of cells that have only half the haploid chromosome number. The diploid chromosome number of *M. sulcinodis* is 56, those of six other *Myrmica* species are between 42 and 48.

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The Isolation of Mossy Fibre Endings from the Granular Layer of the Cerebellar Cortex

When brain tissue is homogenized under carefully controlled conditions, the presynaptic nerve terminals resist disruption and are torn away from their attachments to form discrete particles for which the name 'synaptosomes' has recently been proposed[1-5]. These retain all the morphological characteristics of the intact ending and also account for most of the bound transmitter substances present in the homogenate. The synaptosomes may be isolated by conventional subcellular fractionation procedures and are usually sedimented in the crude mitochondrial fraction.

With cerebellar cortex, the application of these techniques gives rise to two kinds of synaptosomes: (a) large synaptosomes 2–5 μ in diameter[6] derived from the large mossy fibre endings which make synaptic contact with the granule cells of the granular layer[7]; (b) small (0.5 μ diameter) synaptosomes, many of which are believed to be derived from the axo-dendritic endings of the molecular layer, which are the presynaptic terminals of granule cells making synaptic contact with dendrites of Purkinje cells. Such endings can be recognized by the small dendritic spines embedded in their cytoplasm[6]. The large synaptosomes sediment in the crude nuclear fraction (P_1), the smaller synaptosomes in the crude mitochondrial fraction (P_2). The Table shows that although both particulate fractions contain acetylcholine in all four species examined, the distribution of acetylcholine between the fractions shows species variations. In the guinea-pig and rat, fraction P_1 is relatively rich in acetylcholine, whereas in the cat and pigeon this fraction contains much less acetylcholine. The presence of acetylcholine in the large synaptosomes from the guinea-pig was confirmed using a purified preparation obtained from fraction P_1 by a density gradient procedure. Fraction P_1 (prepared as described in the footnote to the Table) was washed once more with 0.32M sucrose and was then suspended in 24% w/v Ficoll in 0.32M sucrose. On centrifuging at 7700 g for 40 min, nuclei and cell debris were sedimented, leaving the large synaptosomes, myelin fragments, and a few mitochondria still in suspension. These were sedimented by diluting the supernatant with an equal volume of 0.32M sucrose and centrifuging at 20,200 g for 40 min. The synaptosomes were separated from most of the myelin and the mitochondria by centrifuging into a discontinuous density gradient consisting of equal volumes of 1.0M and 1.4M sucrose. The intermediate fraction floating between the two layers of sucrose consisted mainly of large synaptosomes, and contained nearly 70% of the acetylcholine of the parent fraction.

This finding suggests that a proportion, at least, of the mossy fibre endings of the guinea-pig and rat are cholinergic, whereas in the cat and pigeon, the cholinergic endings may be largely confined to the population of smaller endings derived from the molecular layer. For the

Distribution of acetylcholine in subcellular fractions of cerebellar cortex of different species

Species	Acetylcholine content					P_1/P_2
	Total recovered ($m\mu$ moles/g)	Homogenate (as % of total recovered in fractions)	P_1	P_2	S_2	
Guinea-pig (3)	4.7	102	41	29	23	1.41
Rat (1)	1.4	140	53	24	23	2.21
Cat (2)	1.9	99	19	45	36	0.42
Pigeon (3)	1.9	88	24	50	26	0.48

Numbers in brackets relate to the number of experiments averaged. The homogenate (10% w/v in 0.32M sucrose) was centrifuged at 1000 g for 10 min to give a pellet (P_1) which was washed once with 0.32M sucrose. The combined supernatants were further centrifuged at 100,000 g for 60 min to give a pellet (P_2) and a supernatant (S_2). Acetylcholine was assayed on a small strip of the dorsal muscle of the leech.

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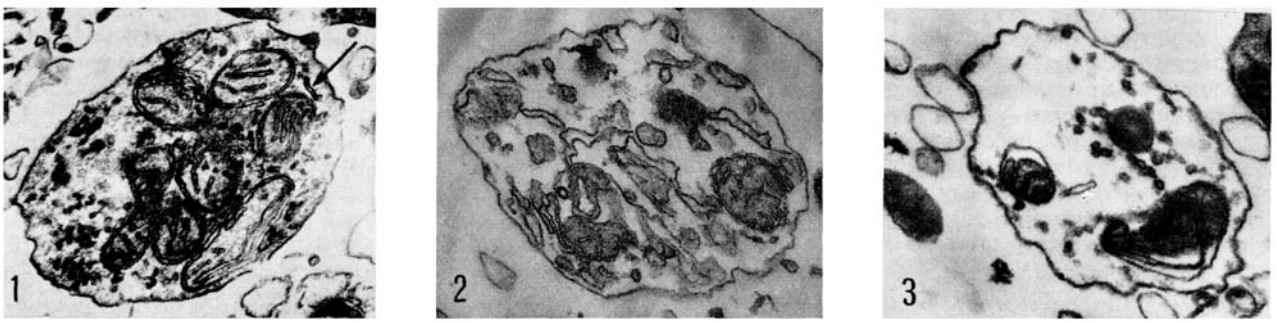

Electron micrographs of three types of giant synaptosome seen in purified preparations from guinea-pig cerebellar cortex. 1, Type similar to those seen in whole tissue sections. 2, Type showing tubules. 3, Type with few vesicles and crenellated margins. Permanganate fixation, Araldite embedding and lead staining, ×35,000.

guinea-pig, rat, and pigeon, but not for the cat, this conclusion is consistent with the recently reported histochemical distribution of cholinesterase in these species[8]. However, in the cat, the electrophysiological evidence[9] indicates that the granule cells with which the mossy fibre endings synapse are not cholinoceptive whereas the Purkinje cells are.

Morphological studies on the large synaptosome fraction in the electron microscope revealed three main types of synaptosome (Figure) with many intermediate forms. Type 1, similar in appearance to the endings seen in whole tissue sections, is commonest in preparations made with good refrigeration and contains numerous synaptic vesicles in its cytoplasm. Short tubules (arrow) are also almost invariably present. Type 2 contains few or no vesicles but many tubules, occasionally seen opening out through the external membrane. Type 3 endings contain few or no vesicles and the external membrane is often crenellated. On slight warming (to 8°) type 2 and 3 synaptosomes are much more frequent, sometimes with considerable disintegration of the external membrane. The tubular structure may in part be derived from autolysing mitochondria, but it seems likely that it results mainly from the intracytoplasmic fusion of the synaptic vesicles. Type 3 synaptosomes may represent those in which the tubular system has evaginated. The intracytoplasmic fusion of vesicles in this way may provide the mechanism for the release of acetylcholine from the vesicles which is known to occur on warming[10]. A reversible fusion and reformation of vesicles could provide a mechanism for transmitter release in vivo[11].

Résumé. Les boutons terminaux des fibres moussues du cervelet ont été separés des petits boutons de la couche moléculaire après homogénéisation et fractionnement. La fusion intracytoplasmique des vésicules synaptiques se produisant sous l'effet de la chaleur indiquerait un mécanisme de libération du médiateur.

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Lokalisation zusätzlicher RNS-Synthese in Trypsin-behandelten Riesenchromosomen von *Chironomus thummi*

Ausgehend von der Annahme[1], dass es Histonmoleküle sind, die in einer differenzierten Zelle durch Blockierung der RNS-Synthese den grössten Teil der genetischen Loci inaktiv halten, versuchten ALLFREY, LITTAU und MIRSKY diese Blockierung durch Trypsin (das spezifisch basische Proteine hydrolysiert) aufzuheben[2]. Tatsächlich gelang es ihnen zu zeigen, dass in isolierten Kalbsthymus-Zellkernen die partielle Entfernung von Histonen die RNS-Synthese auf etwa das 3fache erhöht. Wir haben versucht festzustellen, von welchen Teilen des Genoms diese zusätzliche RNS-Synthese ausgeht.

Als Objekt diente die larvale Speicheldrüse der Zuckmücke *Chironomus thummi*, in deren Riesenchromosomen die Aktivität eines Locus direkt auf Grund einer Strukturmodifikation («puff»), bzw. autoradiographisch feststellbar ist. Die Zellkerne unseres Objektes liessen sich nicht isolieren, und die Explantation ganzer Drüsen in Trypsinhaltige Medien führte nicht zum Ziel; daher wurde eine 0,05%ige Trypsinlösung (gepuffert auf pH 7,3) vermittels einer neuentwickelten Mikroinjektionstechnik[3] direkt in die Zellkerne injiziert. 2–3 h nach der Injektion wurden die Drüsen fixiert, mit Orcein gefärbt und gequetscht. Es zeigte sich eine erhebliche Verstärkung des «puffing» in den Chromosomen. Erstaunlicherweise war diese Ver-

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