tion with other cells[11]. Whether such events may take place in the cell nuclei under our experimental conditions can hardly be decided.

An explanation quite contrary to this may be suggested based on the results of experiments in which *tris* buffer enriched by a low concentration of magnesium was used as suitable for extraction of nuclear proteins[12–14]. The lysine-rich histones, probably their phosphorylated form[15–17], are known to be important for maintenance of chromatin condensation as well in interphase nuclei[18] as in the metaphase chromosomes[19]. However, it can hardly be said whether such an extraction might be responsible for the chromatin decondensation observed by us in the electron microscope. The most reasonable explanation of this phenomenon seems to be the chelating action of *tris* buffer followed by disturbances in inonic medium, as discussed above. The lack of significant changes in the chromatin template activity appeared to be quite understandable. It is well known that special loosening of chromatin fibres is necessary, not being, however, a sufficient condition for initiation of RNA synthesis. The other factors, important in this synthesis, were probably not influenced by our experimental conditions.

Résumé. Des petits fragments de foie de rat ont été maintenus dans un tampon 2 m*M tris* HCl avec ou sans saccharose (concentration final 0,25 *M*). L'incubation du tissus pendant au moins 1 h dans le tampon *tris* cause une décondensation de la chromatine, qui peut être retardée par le saccharose. Les changements morphologiques des types de chromatine n'ont pas l'effet d'incorporer l'uridine dans le noyaux interphasiques du foie.

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Electron Microscopic Observations on the Spinal Projections to the Cerebellar Nuclei in the Cat and Rabbit

Recently it has been suggested that spinocerebellar[1,2] and olivocerebellar tracts[1,3] project their collaterals to the cerebellar nuclei in the cat. Collateral projections of the corticipetal fibres to the cerebellar nuclei were also demonstrated by Golgi studies dealing with branching patterns and modes of termination of nuclear afferents[4,5]. While cerebellar corticonuclear fibres inhibit the activities of nuclei cells[6,7], the collaterals of extracerebellar origin are regarded as excitatory sources for these nuclei cells. Our previous studies using the Nauta and the Fink-Heimer methods have shown that the spinocerebellar tracts (SCTs) send many collaterals to the cerebellar nuclei, especially to the medial and the interpositus nuclei of the cat[2], rabbit[8] and rat[8] (see reference 2 for review). On the other hand, electrophysiological studies indicate that these collaterals are not essentially responsible for producing the excitation of the cerebellar nuclei cells[9–11]. The purposes of the present electron microscope study are to confirm the results of our previous Nauta studies and to investigate the mode of termination of these fibres.

Materials and methods. Either ventrolateral cordotomies or hemisections were made at the cervical levels (C4, C5, C6 and C8) in 7 cats and 2 rabbits. On 2–6 days after operation, the animals were perfused under deep pentobarbital anesthesia with the following fixatives; a mixture composed of 4% paraformaldehyde and glutaraldehyde of various concentrations (either 0.5%, 1.25% or 2.5%) buffered with Millonig's phosphate at pH 7.3 or 7.4. Subsequently, the cerebella were dissected free from the brainstem and small blocks were trimmed from the cerebellar nuclei. After a brief rinse in the above buffer solution, the specimens were placed in 1% osmium tetroxide buffered with Millonig's phosphate, and dehydrated in ethanol and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate. 5 cats and 2 rabbits were used as controls.

Results and discussion. The same results were obtained in the medial and the interpositus nuclei of both sides. In cats and rabbits, a fair number of degenerated boutons were observed synapsing with proximal dendrites and cell bodies of medium-sized and large cells. A small number were seen synapsing with peripheral dendrites of various sizes and dendritic spines. Figure 1 shows that an electron-dense, degenerated bouton containing pleomorphic vesicles forms an asymmetrical synapse with the soma of a large cell of the medial nucleus. Figure 2 also shows that a small, electron-dense, degenerated bouton synapses with the soma of a large cell of the medial nucleus. The latter

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is markedly dense and shrunken, but vesicle-like structures can be recognized. Previous Golgi studies[4, 5, 12] show that fibres of extracerebellar origin terminate on the cerebellar nuclei cells, coursing or climbing along the dendrites and the surface of cell bodies. Correspondingly, climbing-like terminals were frequently encountered under the electron microscope. Figure 3 illustrates the degeneration of a large and elongated fibre terminal. This electron-dense bouton contains some vacuoles and altered synaptic vesicles, making 4 synaptic contacts with the primary dendrite of a large cell of the medial nucleus. Boutons exhibiting filamentous degeneration were observed. One

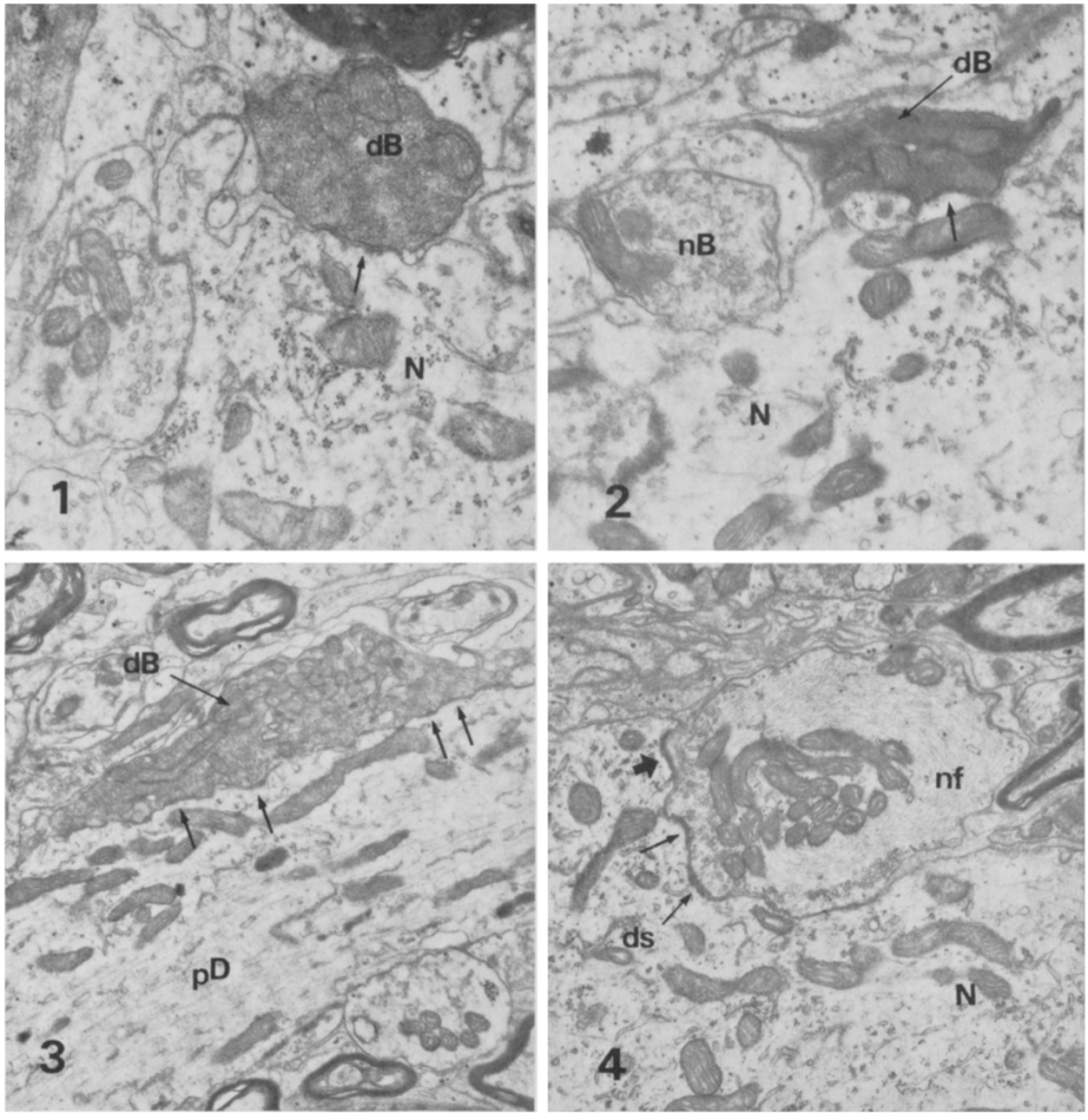

Fig. 1. An electron-dense, degenerated bouton (dB) making an asymmetrical synapse (arrow) with the soma (N) of a large cell of the medial nucleus in the rabbit. Pleomorphic vesicles are clearly seen. Side contralateral to hemisection at C5, 4 days postoperatively. ×14,600.

Fig. 2. An electron-dense, degenerated bouton (dB) synapsing (arrow) with the soma of a large cell (N) of the medial nucleus in the rabbit. Synaptic vesicle-like structures are seen within the bouton. A normal bouton with less dense matrix is located in the vicinity (nB). Side contralateral to hemisection at C5, 4 days postoperatively. ×18,200.

Fig. 3. An electron-dense, degenerated bouton (dB) synapsing with the primary dendrite (pD) of a large cell of the medial nucleus in the cat. It makes 4 synaptic contacts (arrows) along the dendrite. Side contralateral to hemisection at C5, 4 days postoperatively. ×12, 900.

Fig. 4. A filamentous degenerated bouton synapsing with the perikaryal profile of a large cell (N) of the medial nucleus in the cat. Spherical synaptic vesicles and large numbers of neurofilaments (nf) are seen. Large arrow indicates a synaptic specialization from which the presynaptic component has been detached. A desmosome-like contact is also seen (ds). Side contralateral to a lesion in the lateral funiculus at C5, 5 days postoperatively. ×12, 900.

of them in Figure 4 makes an asymmetrical synapse with a large cell of the medial nucleus; the whole bouton is swollen and filled with a coiled mass of neurofilaments. By invagination of developed glial processes, part of this bouton is detached from its contact region, and the postsynaptic membrane thickening remains in the corresponding site (Figure 4, large arrow). In the present study it was found that the boutons derived from SCT fibres largely formed asymmetrical synapses with small-sized dendrites, dendritic spines and cell bodies, and a few formed symmetrical synapses on the cell bodies with wide synaptic clefts.

According to recent electrophysiological studies, excitatory responses with long latencies were recorded in the medial and the interpositus nuclei following stimulation of peripheral limb nerves[9-11], cutaneous mechanoreceptors[9,10], and hindlimb muscle afferents[11], and those with short latencies were rarely recorded following stimulation of the ventrolateral funiculus at C3[10]. Therefore, it is concluded that major input to the cerebellar nuclei is derived from the lateral reticular nucleus or the inferior olive, rather than from the spinal cord. The present observations apparently show that the fibres from the spinal cord terminate directly upon the dendrites and somata of large cells in the cerebellar medial and interpositus nuclei. However, it is not possible to estimate exactly the number of SCT fibre terminals in these nuclei.

Zusammenfassung. Bei Katze und Kaninchen wurden Projektionen vom Rückenmark zu den Kleinhirnkernen elektronenmikroskopisch untersucht und nach Läsionen des Halsmarkes wurden viele degenerierte Endknöpfe der Kollateralen des Tractus spinocerebellares in den Nuclei medialis und interpositus gefunden.

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Synthesis of Nuclear and Mitochondrial DNA in Rat Liver After Injection of the Carcinogenic Compound Diethylnitrosamine

Diethylnitrosamine (DENA) is carcinogenic in many animal species[1] and can induce liver tumors either after prolonged feeding, in the diet[2], or after single administration[3]. Early modifications of nuclear DNA (nDNA) synthesis in rat liver have been observed after injection of dimethylnitrosamine (DMNA)[4], and late effects of DENA on rat liver nDNA synthesis have been analyzed autoradiographically by RAJEWSKI[5]. Although the alkylation of nucleic acids by DENA in rat tissues is less severe than that of similar amounts of DMNA, the basic mechanism of action is the same in both cases and involves the appearance of N-7 alkylated guanine[6]. This similitude incited us to look for possible differences in the effects of DENA on the synthesis of rat liver nDNA and mitochondrial DNA (mtDNA)[7], the probability of which is strengthened by the observation of WUNDERLICH et al.[8] that liver mtDNA

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Specific radioactivity, in dpm/mg DNA, of nDNA and mtDNA after injection of DENA

Time (h) after injection	nDNA			mtDNA		
	DENA	controls	DENA/controls	DENA	controls	DENA/controls
1	980	1800	0.55	89.150	52.500	1.70
	2300	5700	0.41	25.280	15.140	1.67
2	2170	4430	0.49	47.650	59.370	0.80
	715	1300	0.55	31.620	38.890	0.81
4	653	1970	0.33	46.140	33.570	1.37
	865	2360	0.36	49.506	39.470	1.25
6	644	913	0.70	32.940	43.610	0.75
	940	1100	0.92	—	—	—
12	630	3670	0.17	34.965	51.708	0.67
	1200	3560	0.34	12.590	37.780	0.33
24	5830	2770	2.1	11.230	59.000	0.19
	2320	1020	2.3	12.070	48.000	0.25
30	7650	1250	6.1	—	—	—
	4500	2180	2,0	7.400	24.760	0.30
48	48.890	1700	28.7	60.800	66.580	0.91
	20.230	4355	4.6	—	—	—

Results of 2 separate experiments; 5 rats are used per experiments.