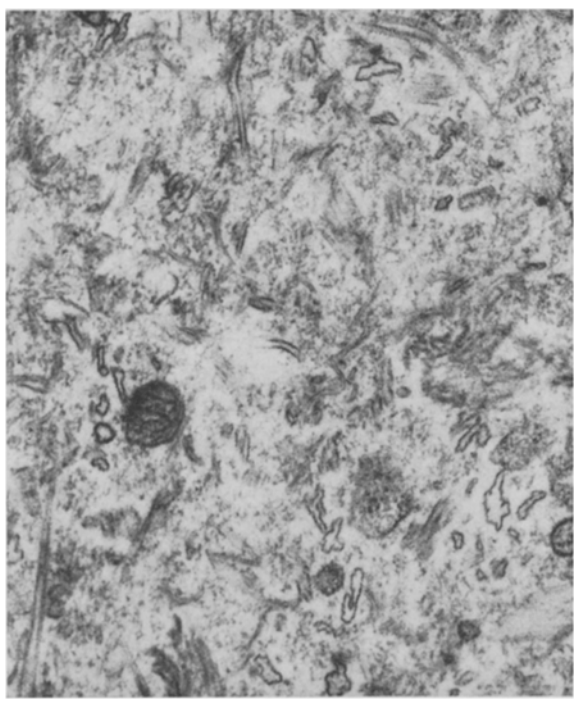
giving up completely the above concept, we should like to suggest the following. First of all, it should be emphasized that only one of the two enzymes involved in this pathological process, namely galactorecebroside β-galactosidase, is a lysosomal one. Lipid sulfotransterase, on the other hand, is not lysosome-bound but, like other transferases, it is closely associated with the smooth-surfaced endoplasmic reticulum[9]. One may suggest that, in the course of Krabbe's disease, deficient or absent activity of galactocerebroside β-galactosidase is responsible mainly for the accumulation of galactocerebrosides within membrane-bound vacuoles or bodies of lysosomal nature; lowered lipid sulfotransferase activity, in turn, accounts for the deposition of particulate galactocerebroside inclusions within the cytoplasmic matrix proper. Secondly, liberation of originally intralysosomal galactocerebroside deposits into the ground cytoplasm as a consequence of membrane labilization and lysosome leakage, must also be taken into consideration. Finally, it should be mentioned that, in Krabbe's disease, membrane-bound lysosomal structures may be only secondarily involved in the process of storage, as has been supposed in some other sphingolipidoses[11]. This would imply, of course, an initial extralysosomal stage of the deposited substances. Moreover, one would be compelled to assume that lysosomal hydrolases may be operative, at least to some extent and/or for short intervals, also out of the lysosome system in normal cell metabolism. Up to the present, no direct evidence exists for any of these hypotheses. However, we feel that one or more of them may be verified in the future. Be that as it may, the general concept of inborn lysosomal storage diseases evidently requires reconsideration and/or completion. In its present form, it does not satisfactorily explain all morphological findings thus far obtained in human GLD as well as in various other storage diseases.



Cytoplasmic portion of an inclusion-bearing cell in GLD brain biopsy material. Note polygonally shaped and needle-like profiles representing transversally and longitudinally sectioned deposits which obviously lack any membranous demarcation against the ground cytoplasm. ×30,000.

Zusammenfassung. Bei der Krabbeschen Leukodystrophie können die charakteristischen Zytoplasmaeinlagerungen sowohl innerhalb als auch ausserhalb membranbegrenzter lysosomaler Kompartimente vorkommen. Es wird versucht, für diesen Befund einige plausible Erklärungen zu geben. Ausserdem wird hervorgehoben, dass das allgemeine Konzept der angeborenen lysosomalen Speicherkrankheiten in seiner bisherigen Form nicht ausreicht, alle morphologischen Beobachtungen bei den verschiedenen Thesaurismosen befriedigend zu deuten.

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Fractionation and Characterization of Cell Nuclei from Rat Neocortex by Protein-DNA Ratios[1]

Several different isolation and fractionation methods have been developed for brain nuclei[2–4], but criteria for their differentiation are still controversial. The protein/DNA ratios found in the literature vary remarkably[4–6], and no information can be found on the problem of reproducibility. For our procedure we adopted a slightly modified method of Burdman[2], which gave reproducible results when a strict time table was observed. Since the morphological criteria described in the literature are clearly insufficient, we have characterized our nuclei by chemical parameters, and these will be compared with the findings of other authors.

Methods: (Times in parenthesis indicate min between the death of the animal and the next step of the procedure). 2 male rats (300–400 g) used for each experiment are anaesthesized and killed by decapitation. All further steps are carried out at 0–4°C. The combined neocortex (ca. 1.2 g) is homogenized (15 min) by hand (15 strokes) with 0.32 *M* sucrose containing 1 m*M* $MgCl_2$ and 1 m*M* KH_2PO_4, pH 6.5 (w/v 1:15), with a Teflon pestle in a Potter homogenizer. The homogenate is diluted to 50 ml with homogenization medium and centrifuged for 10 min at 900 g_{av}

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(23 min). This crude nuclear pellet is then further purified by a Chauveau procedure as modified by BURDMAN[2] (37 min). The transparent pellet is resuspended in 3.5 ml of 2.2 *M* sucrose and 1 ml of this suspension is layered on each of two identical gradients, based on the gradient of BURDMAN[2]. The gradients (6 ml tubes), prepared 30 min before use, consist of (from bottom to top): 1 ml 2.8 *M* sucrose as a cushion, 1.5 ml 2.47 *M* sucrose, 1 ml nuclear suspension (2.2 *M*) and 2 ml 1.8 *M* sucrose overlay. The cushion and overlay are not in the original BURDMAN[2] procedure. The gradients are centrifuged in a Christ Omikron centrifuge (130 min) at 63,500 g_{av} for 45 min in the rotor (Christ) No. 9630. The smaller nuclei are collected at the 2.8 *M* cushion, and the larger nuclei on top of the 2.47 *M* layer. Protein is determined after a Schneider extraction according to LOWRY et al.[7] and DNA by the BURTON[8] method.

Results and discussion. The method of LØVTRUP and MCEWEN[4] is said to give distinct astrocyte and neuronal fractions. Since we were not able to characterize the different groups of nuclei unequivocally, we decided to describe our two fractions from rat brain merely by distinguishing between large and pale, and small and dark nuclei. The upper band, containing essentially larger and paler nuclei (probably mostly neurons and astrocytes), is contaminated with 20–30% of smaller nuclei, while the lower layer consisting mainly of much smaller and darker nuclei (probably mostly oligodendrocytes and microglia) has a contamination of 10–25% of larger nuclei. The protein/DNA ratios for these 2 nuclear populations were determined in 8 experiments. The determinations were made on 2 parallel gradients in each experiment (Table I), and are quite consistent.

Table II shows the considerable variety of methods used for the fractionation and purification of nuclei, and for the quantitative determination of DNA and protein. Since we believe that the protein/DNA ratios depend on the procedure by which the nuclei have been isolated, we have divided Table II into 2 parts. The first group of data gives protein/DNA values which were determined on total nuclei of different organs and animals. These nuclei were purified by one high molar sucrose centrifugation ('Chauveau'-type purification) and were not further fractionated. The ratios for brain nuclei of LØVTRUP and MCEWEN[4], BURDMAN and JOURNEY[5] and HADJIOLOV et al.[9] in this group coincide very well. Yet their values differ by a factor 2–3 from our values determined for total rat brain cortex nuclei after the Chauveau procedure as modified by BURDMAN[2] (Table II). In order to test our chemical determinations, the protein/DNA ratio of rat liver nuclei was determinad after isolation by the method of TENG et al.[10] (Table II). Our value (4.1) coincides very well with the findings of TENG[10] (4.2), indicating that our determinations do not give too low values.

The second part of Table II (fractionated nuclei) shows that our results are in remarkably good agreement with the findings of KATO and KUROKAWA[11]. They determined protein after ethanol/ether extraction of 2 nuclear fractions which are similar to ours in their composition. The ratio we found for total nuclear fractions is higher (4.9) than the ratio of the upper fraction of our gradient, consisting mainly of larger nuclei (3.9). This difference might be partly due to purification or to loss of nuclear material during the second high molar fractionation. The great discrepancy between the ratios of LØVTRUP and MCEWEN[4], BURDMAN and JOURNEY[5], HADJIOLOV et al.[9] and our values for total nuclei and fractionated nuclei (Table II) might in part be explained by the circumstance that these authors do not mention extraction of lipids or peptides prior to protein determination. LØVTRUP (personal communication) determined protein directly from the whole nuclei.

Thus it appears that protein/DNA ratios are extremely sensitive to the type of extraction procedure used, and that it is not yet possible to say which methods best conserve characteristics of the intact nucleus. High protein/DNA ratios might indicate cytoplasmic contamination of the nuclei, whereas low ratios might indicate losses of nuclear material during the extraction procedure.

Zusammenfassung. Die Kerne des Rattenneocortex wurden mit einer nach BURDMAN modifizierten Methode auf einem diskontinuierlichen Saccharosegradienten in zwei Populationen aufgetrennt. Die Kerne werden durch das Protein/DNA-Verhältnis charakterisiert: kleine Kerne 1.5; grosse Kerne 3.9. Die morphologische Differenzierung im Phasenkontrastmikroskop erwies sich als ungenügend.

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Table I. Protein/DNA ratios of rat neocortex nuclei after fractionation on a discontinuous sucrose density gradient

	Tube 1		Tube 2	
Exp. No.	small nuclei	large nuclei	small nuclei	large nuclei
1	2.0	4.1	2.1	4.3
2	1.4	3.7	1.5	3.8
3	1.3	3.8	–	–
4	1.2	3.5	1.1	3.6
5	1.6	4.6	1.4	4.4
6	1.5	4.3	1.4	4.1
7	1.5	3.6	1.2	3.7
8	1.5	3.5	1.6	3.7
Mean	1.5	3.9	1.5	3.9

Small nuclei collected on 2.8 *M* sucrose layer, large nuclei collected on 2.47 *M* layer. Results from 2 parallel gradients in each of 8 experiments are given.

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Table II. Comparison of protein/DNA ratios of liver and brain cell nuclei

Author	Animal and organ	Purification steps (*M* sucrose)	Fraction	Protein/DNA	Chemical tests
high molar Chauveau-type purification					
Burdman and Journey[5]	Rat brain	2.06	total nuclear suspension	17.7 without Triton 5 with Triton	Protein: Lowry[7] DNA: diphenylamine
Løvtrup and McEwen[4]	Rat brain	2.06	nuclear suspension	14.4 without Triton 9.1 with Triton	Protein: Lowry[7] without lipid extraction DNA: Hoff-jorgensen[13]
Hadjiolov et al.[9]	Cat brain cortex	1.8	final nuclear fraction	14.9 with Cemulsol NPT 12	Protein: Lowry[7] DNA: Tsanev and Markov[14]
Maggio et al.[6]	Guinea-pig liver	2.2	total nuclei	3.3	Protein: nesslerization DNA: diphenylamine
Teng et al.[10]	Rat liver	2.4	total nuclei	4.2	Protein: Lowry[7] DNA: fluorometry
Olpe et al.[12]	Rat liver	2.4	total nuclei	4.1	Protein: Lowry[7] after Schneider[15] extraction DNA: Burton[8]
Olpe et al.[12]	Rat brain cortex	2.2	total nuclei	4.9	Protein: Lowry[7] after Schneider[15] extraction DNA: Burton[8]
High molar Chauveau-type purification + fractionation					
Kato and Kurokawa[11]	Guinea-pig cortex	2.3	PL neuronal fraction	2.6	Protein: Lowry[7] after removal of lipids
		2.6	Ps oligodendroglia fraction	1.4	DNA: fluorometry
Olpe et al.[12] (Table I)	Rat brain cortex	2.2	large nuclei	3.9	Protein: Lowry[7] after Schneider[15] extraction DNA: Burton[8]
		2.47	small nuclei	1.5	