ears, and pink snouts produced by the Mi^{wh} gene serve as characteristic markers for $Lc/+$ heterozygotes, since Mi^{wh} is linked on the same chromosome with *Lc*.

The overall size of the adult lurcher ($Lc/+$) brain appears to be similar to that of an adult normal ($+/+$) brain from the same strain. However, there is a noticeable reduction in the size of the lurcher cerebellum, which is approximately one-half the size of the normal cerebellum (Figures 1 and 2). In addition to the reduced size, the lurcher cerebellum shows lobular defects. All of the lobules are usually present, but most are narrower and flatter than their normal counterparts. The uvula vermis, in particular, is quite narrow and extends further laterally than is the case in the normal cerebellum.

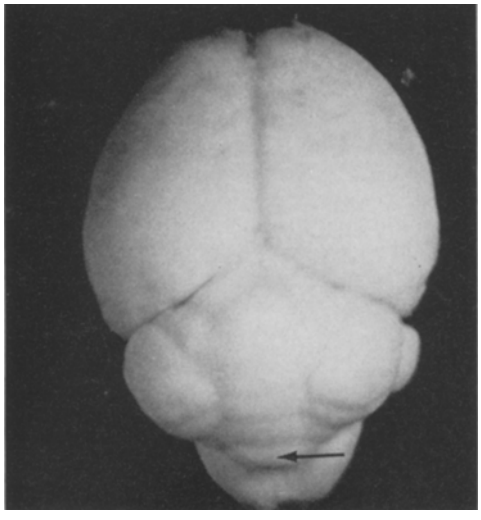

Fig. 1. Dorsal view of normal ($+/+$) adult brain. Arrow indicates uvula vermis. ×6.

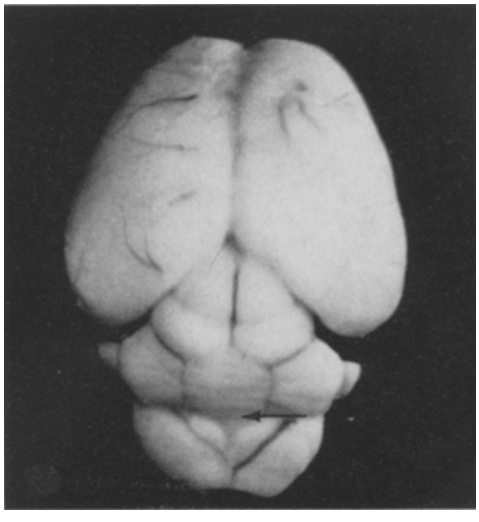

Fig. 2. Dorsal view of lurcher ($Lc/+$) adult brain. Arrow indicates slender uvula vermis. Note also the small size of the cerebellum, the exposed roof of the 4th ventricle, and the prominent collicles. ×6.

The reduced cerebellar hemispheres do not adequately cover the anterior portion of the medulla; as a result, the roof of the 4th ventricle is usually exposed in a dorsal view of the lurcher brain. The collicles appear to be more prominent in lurcher than in normal mice, possibly because of the less acute angle formed by the cerebral hemispheres. The ventral surface of the brain does not show any obvious defects.

Gross defects in the lurcher brain, particularly the cerebellum, are exhibited as early as 3 days after birth. At this time the uvula vermis is prominent and oval in shape in the normal brain, but narrow and elongated in the lurcher. The overall reduction in the size of the cerebellum becomes obvious at approximately 5 days after birth.

Discussion. The cerebellar hypoplasia in lurcher adult mice is similar to that described in the reeler (*rl*) mutant[2,3]. As in reeler, this reduction in size is constant from animal to animal, although a thorough study of lurcher cerebellar histogenesis is warranted before comparisons can be made with other cerebellar mutants. Since the lurcher individuals in our colony are descendants of a translocation stock, it is also necessary to determine whether or not this genetic background exerts any effect on the phenotypic expression of the lurcher gene. However, preliminary observations on the brain of a lurcher individual believed not to be carrying the translocation show no gross morphological differences from those carrying the translocation.

It is probable that the defects observed in the collicles and medulla result from the failure of the cerebellum to grow properly, although primary malformations may also occur in these regions. Since defects in the shape of certain $Lc/+$ cerebellar lobules become apparent by the third day postnatally, well before any behavioral characteristics are manifested, the early stages of cerebellar histogenesis are accessible for study in this mutant. Moreover, $Lc/+$ neonates are distinguishable from their normal ($+/+$) littermates by means of color characteristics even before the gross cerebellar defects can be detected. This mutant, therefore, should serve as an excellent model in which to study the early etiology of cerebellar dysgenesis.

Résumé. Le cervelet des souris hétérozygotes de la mutation «lurcher» (*Lc*) présentent des anomalies. Il est réduit et déformé, en particulier l'uvula vermis. On peut détecter ces anomalies chez le nouveau-né de 3 jours avant l'apparition des aberrations du comportement.

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Isolation of Highly Purified Glomerular Complexes from Rabbit Cerebellum

Gentle disruption of brain tissue has been increasingly employed during the development of methods for large scale isolation of neuronal and glial cells[1-5]. Softening of the tissue through incubation of slices at 37 °C, followed by passage of tissue through nylon mesh of decreasing pore size, results in the recovery of larger and more intact cellular units than are generally obtained by homogenization in cold sucrose media.

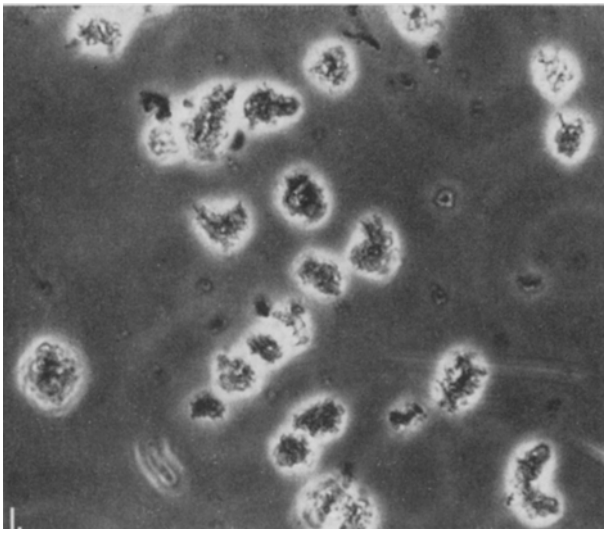
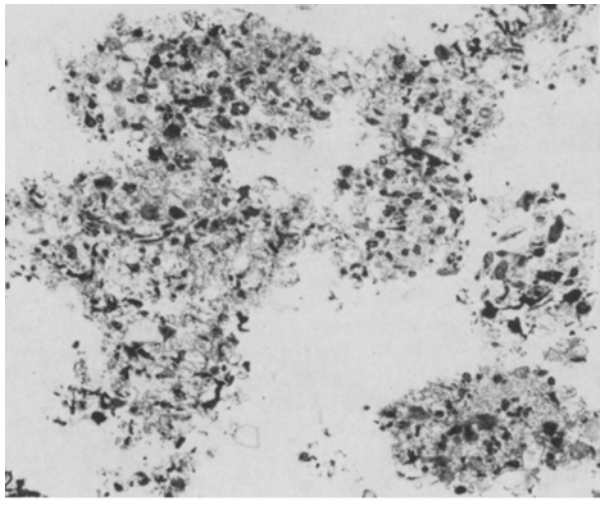


Fig. 1. Phase contrast light micrograph of the purified glomerular fraction from rabbit cerebellum. There are no nuclei intact, cells or blood capillaries among the glomerular complexes.

Isolation of cells from cerebellum has proved more difficult, partly due to the very heterogeneous neuronal cell population. A few reports on the successful isolation of Purkinje cell perikarya and granular cells have appeared during the last years[6–9]. However, the heterogeneous fraction recovered from cerebellum[7] when a method for isolation of glial cells from cerebral cortex was applied[3], could not be identified as a glial fraction. With the exception of a few astrocytes and blood capillaries, the 'cerebellar glial' population consisted of rounded, generally non-nucleated tissue fragments when observed in the light microscope. The only striking feature was the homogeneity with respect to size and shape of this fragment population. Further examination of this 'cerebellar glial' fraction with transmission electron microscopy revealed that it consisted mainly of apparently intact glomerular complexes. Using an additional purification step it has been possible to obtain a 'glomerular' fraction of very high purity.

The techniques employed was essentially as described by BLOMSTRAND and HAMBERGER[3]. The pooled cerebella from 5–6 adult white rabbits (body weight 1.5–2.0 kg) were chopped into 0.4 mm slices and incubated for 30–45 min at 37 °C with shaking, in a medium (2 ml medium/g) containing 35 m*M* *Tris*-HCl buffer, and 5 m*M* Na-phosphate buffer, pH 7.6, 120 m*M* NaCl, 5 m*M* KCl, 2.5 m*M* $MgCl_2$, 20 m*M* glucose and 2% Ficoll (Pharmacia Fine Chemicals, Uppsala, Sweden). The tissue was disrupted by passage through nylon mesh mounted on a cut plastic syringe. The disrupted tissue was diluted with isolation medium (0.32 *M* sucrose, 50 m*M* NaCl, 10 m*M* *Tris*-HCl, pH 7.4, 0.5 m*M* EDTA and 2% Ficoll). The suspension was sieved through nylon mesh with descending pore size, 500, 200, 100 and 50 μm, and a final sieving through two layers of 50 μm mesh. All steps were performed either in a cold room or on ice. The filtrate was centrifuged for 5 min at 150 *g* and the supernatant decanted. The pellet was fractionated on a discontinuous Ficoll gradient which was centrifuged for 110 min at 80000 *g*. The 150 *g* pellet was resuspended to 5 ml with the isolation medium and mixed with Ficoll to a final Ficoll concentration of 24.6%. This suspension was the bottom layer of the gradient tube. Equal volumes of 19.1% and 15.7% Ficoll, respectively, were added as overlayers in the gradient. These solutions were also made up in isolation medium. After centrifugation, the layer between 19.1% and 15.7% was collected with a Pasteur pipette, diluted with large volumes of 0.32 *M* sucrose, 10 m*M* *Tris*-HCl, pH 7.4 and centrifuged at 15.000 *g* for 15 min. The pellet, the 'crude glomerular fraction', was



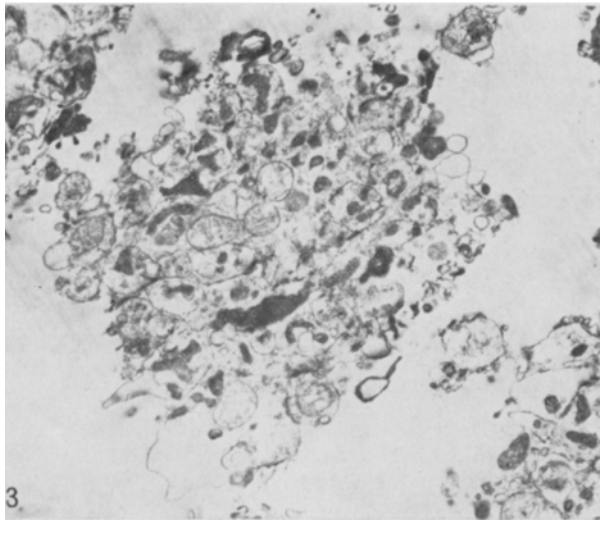


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Fig. 2. Electron micrograph of the purified glomerular fraction. The glomerular complexes are lacking contaminating structures such as myelin and cellular fragments including nuclei.
Fig. 3. Higher magnification of an isolated glomerular complex showing the central mossy fibre surrounded by synaptic terminals from granule cells and, at the periphery, from Golgi cells.

resuspended by gentle hand homogenization in the sucrose solution and layered on top of a 1.4/1.2/0.8 *M* discontinuous sucrose gradient and centrifuged for 1 h at 80,000 *g*. The layer between 1.2 and 1.4 *M* sucrose contained the bulk of the material which was a virtually pure glomerular fraction (Figure 1). Cells and nuclei were found in the pellet and finely granular material seen in the 1.2/0.8 *M* interphase. The final glomerular fraction was diluted with 0.32 *M* sucrose and pelleted as described above.

The ultrastructural examination was made after fixation of either the suspensions or the pellets with Karnovsky's fixative, followed by dehydration in ethanol, embedding and sectioning as previously described[10].

E.M. examination showed that approximately 70% of the 'crude glomerular fraction' consisted of glomerular profiles. The glomeruli were well preserved, i.e. the central mossy fibre rosette contained neurotubuli, filaments, endoplasmic reticulum, numerous mitochondria and clusters of synaptic vesicles. They were surrounded by a sheath of pocesses from granule cells and Golgi cells. Delicate lamellar astrocytic pocesses outlined some of the glomeruli, Most of the other material in the crude glomerular fraction consisted of small cells, mostly granule cells and astrocytes, cell nuclei, with or without a rim of cytoplasm, and blood capillaries. The final glomerular fraction consisted of well-preserved glomeruli as described above (Figures 2 and 3). Occasionally, scattered debris could be observed. The purity of the fraction was determined from electron micrographs, from which the structures were cut out and weighed. The percentage of the fraction which consisted of glomeruli was estimated as 93.6 $\pm$ 4.0 (S.D.) from 5 different preparations.

The average yield of glomerular protein from a purified fraction of rabbit cerebellum (wet weight ~1.1 g) was 0.1 mg as determined with the method of LOWRY et al.[11]. Between 800,000 and 900,000 glomeruli per cerebellum were isolated which gives a protein content of 12×10^{-11} g per glomerulus.

The present method offers the possibility to isolate a highly purified fraction of morphologically well preserved glomerular complexes from rabbit cerebellum. The fraction has particular advantages for biochemical and neuropharmacological studies, as glomeruli are of known origin and function[12]. However, the complex composition of the glomerulus, containing several pre- and postsynaptic compounds, as well as glial cell processes makes them rather unique as synaptosomes, but may also limit the usefulness of this fraction for functional studies, until further subfractionation methods have been developed. Since the disruption of the tissue is carried out without homogenization, the possibility to preserve larger intact particles is improved. Previous procedures for cerebellar tissue subfractionation employed homogenization of the fresh tissue. ISRAEL and WHITTAKER[13] isolated the central core of the glomerular complex, i.e. the mossy fibre endings, by sucrose gradient separation of a crude nuclear fraction. HAJOES et al.[14] and TAPIA et al.[15] have described in a careful study of subcellular fractionation of rat cerebellum homogenates, the isolation of a fraction which consisted of glomerular complexes to a purity of at least 50%. The higher purity of the fraction obtained by our method is partly outweighed by the more time-consuming isolation procedure. However, a preliminary biochemical characterization showed good retention of lactic dehydrogenase and active accumulation of amino acids by the isolated glomeruli which indicates their usefulness for functional studies[16].

Zusammenfassung. Es wird eine neue Methode zur Präparation der sogenannten «glomerulären Komplexe» aus Kaninchen-Kleinhirn beschrieben, deren komplexe innere Struktur weitgehend intakt bleibt. Mit Hilfe der quantitativen elektronenmikroskopischen Methode wurden über 90% intakter Glomeruli errechnet.

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Acid Phosphatase in Golgi Vesicles of Osteoblasts

The collagen producing cells have been studied in detail by various workers[1-3]. These cells contain very large ribosomal complexes[4] related to the assembly of long α-chains of protocollagen. The latter have been shown to be present in the cisternae of the endoplasmic reticulum (ER) and it has been shown that this material is then transferred to the Golgi complex[2,4-6]. In addition to collagen these cells also synthesize and secrete other components of the matrix and Golgi complex of odontoblasts has been shown to be involved in the synthesis and secretion of glycosaminoglycans[7]. The Golgi complex in osteoblasts is quite large and consists of many flat saccules with distended ends (Figure 1). As shown previously[3,4] a substructure can be made out in these distentions. Most often it is in the form of randomly coiled filaments (*). In the Golgi region, there are also present many oblong or elongated vesicular structures. The filaments present in these vesicles are so aligned that longitudinal striations can be clearly made out (→). These filaments do not show any banding pattern.

For the present study in addition to routine preparations described above, some of the material (calvaria of 15-day-old chick embryos, White Leghorn) was incubated for acid phosphatase[8] after fixation (30 min) in 1% glutaraldehyde in 0.1 *M* cacodylate, pH 7.4 and prepared for electron microscopy. The material incubated for the shortest period (20 min) was found to be most useful as the reaction product did not obscure the morphological