Lysosomal hydrolases in neuronal, astroglial, and olidodendroglial enriched fractions of rabbit and beef brain

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Abstract Arylsulfatases A, B, and C, β-galactosidase, and acid phosphatase were assayed in neuronal, astroglial, and oligodendroglial fractions isolated from adult rabbit and beef brains. The specific activities of all acid hydrolases were lower in beef cells compared to rabbit cells. The lysosomal enzymes of the rabbit neuronal fraction showed 10–25 times higher activities than the oligodendroglial fraction and 5-fold higher activities than the astroglial fraction. In beef brain, the specific activities of these enzymes were similar in oligodendroglia and astrocytes but 4–10 times lower than in neurons. The low activity of arylsulfatase A and β-galactosidase in oligodendroglial cells may suggest that the low turnover of cerebroside and sulfatide in myelin may be regulated in part by the enzymes that catalyze their degradation.—**Freysz, L., A. A. Farooqui, Z. Adamczewska-Goncerzewicz, and P. Mandel.** Lysosomal hydrolases in neuronal, astroglial, and oligodendroglial enriched fractions of rabbit and beef brain. *J. Lipid. Res.* 1979. **20:** 503–508.

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Since de Duve et al. (1) first characterized the rat liver lysosomes, these subcellular organelles and their associated enzymes have been reported to occur in many organs and tissues (2). The presence of lysosomes in the nervous system has been demonstrated by electron microscopic, cytochemical, and biochemical methods (3). The mammalian central nervous system contains three main types of cells, namely neurons, astroglia, and oligodendroglia which can be separated from one another by several techniques (4–12). Although considerable information is available on the morphology and the nucleic acid and lipid compositions of neurons, astroglia, and oligodendroglia of the brain, very little is known about the lysosomal hydrolases of the cell types, except for some neuronal glycosidases (13–15).

Several studies have indicated that the acid hydrolases are more concentrated in the neuronal regions compared to non-neuronal regions of the brain (3). Sinha and Rose (16) also reported a similar enrichment of acid hydrolases in neuronal perikarya compared to the neuropil fraction of adult rat brain. Studies made by Raghavan, Rhoads, and Kanfer (17), on the other hand, did not show any significant differences in the distribution of acid hydrolases in the neuronal and glial cell fractions of the young rat brain. Earlier work from this laboratory (18) has indicated that the activities of arylsulfatases A and B and β-galactosidase were several fold higher in neuron–glia mixed primary cell culture than primary astrocyte cell culture. In the present communication, we report our results on the distribution of lysosomal acid hydrolases in the neuronal, astroglial, and oligodendrial-enriched fractions of rabbit and beef brain.

MATERIALS AND METHODS

p-Nitrophenyl sulfate, *p*-nitrocatechol sulfate, and *p*-nitrophenyl β-galactoside were obtained from Sigma Chemical Co., (St. Louis, MO). *p*-Nitrophenyl phosphate was purchased from Merck, Darmstadt, West Germany. Ficoll was obtained from Pharmacia, Uppsala. All other chemicals used in the present study were of analytical grade.

Isolation of neuronal, astroglial, and oligodendroglial cell fractions from adult rabbit and beef brain

Neurons and astrocytes were isolated from gray matter of the brain cortex by the method of Iqbal and Tel-

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lez (11) with slight modifications. The gray matter was chopped with a razor blade in a medium consisting of 10 mM K^+-phosphate buffer, pH 6.0, containing 10% glucose, 10% fructose, and 5% Ficoll (3 ml/g). After incubation for 1 hr at 37°C in a shaking bath the cells were dissociated by gently pressing the tissue two times with the help of a glass rod through a nylon cloth of 110 mesh under slight vacuum. The homogenate was then filtered three times through a stainless 200 mesh cloth under slight vacuum. The homogenate was brought to a volume of 10 ml/g with the medium and diluted with an equal volume of the same medium containing 50% sucrose. The homogenate was centrifuged on a gradient in a SW27 rotor at 6500 *g* for 10 min. From the bottom to the top, the gradient contained 5 ml of 50% sucrose, 5 ml of 45% sucrose, 5 ml of 40% sucrose, 5 ml of 35% sucrose, and 18 ml of homogenate. All sucrose solutions were made in the medium and adjusted to pH 6.00. The neurons were collected at the 45–50% sucrose interface, and the astrocytes at the 35–40% sucrose interface. The astroglial fraction was diluted 2-fold with the medium and layered on a gradient consisting of 8 ml of 40% sucrose, 8 ml of 35% sucrose, and 22 ml of homogenate. The gradient was centrifuged in a SW27 rotor at 6500 *g* for 10 min. The astrocytes were recovered at the 35–40% sucrose interface.

Oligodendroglia were isolated from the white matter of the cerebral hemispheres. The cell homogenate was prepared under the same conditions as those used for neurons and astrocytes. The isolation of oligodendroglial cells was performed on the same gradient and under the same conditions as those used for neurons and astroglial cells. The oligodendroglia were recovered at the 40–45% sucrose interface.

The morphological integrity of the isolated cells was examined under a phase microscope. The three cell fractions were diluted slowly with 4 volumes of medium and centrifuged at 900 *g* for 10 min. The pellets were homogenized in 50 mM Tris-HCl buffer, pH 7.4, and dialyzed overnight against 100 volumes of the same buffer. These dialyzed preparations were used for protein and enzymatic determination.

Assay of lysosomal hydrolases

Arylsulfatases A and B were assayed by the method of Baum, Dodgson, and Spencer (19) using *p*-nitrocatechol sulfate as substrate, with slight modification. The assay mixture consisted of 0.8 ml of the Baum et al. (19) reagents and 50–200 μg of enzyme protein in a total volume of 1 ml. For the determination of neuronal and astroglial enzyme activities, tubes were incubated at 37°C as described by Baum et al. (19). Since the protein concentration and the activities of arylsulfatases were very low in the oligodendroglial cell fraction, the latter was incubated for 5–10 hr. The reaction was stopped by the addition of 4.8 ml of 0.5 M sodium hydroxide and the liberated *p*-nitrocatechol was determined spectrophotometrically at 510 nm. The activities of both oligodendroglial enzymes were linear with time up to 10 hr.

Arylsulfatase C was assayed by the method of Clendenon and Allen (20) using *p*-nitrophenyl sulfate as substrate.

β-Galactosidase was assayed by the method of Gatt and Rapport (21) using *p*-nitrophenyl β-galactoside as substrate. The reaction was terminated by the addition of 0.5 ml of 0.5 M sodium hydroxide and the liberated *p*-nitrophenol was determined as described above.

Acid phosphatase. The assay mixture consisted of 50 mM sodium citrate buffer, pH 4.8 (0.5 ml); 55 mM *p*-nitrophenyl phosphate (50 μl), and 50 μl of enzyme protein (50–200 μg) in a total volume of 0.6 ml. The tubes were incubated for 15–30 min at 37°C and the reaction was stopped by the addition of 2.0 ml of 0.5 M sodium hydroxide. The yellow color of *p*-nitrophenol was determined spectrophotometrically at 405 nm. It should be noted here that the assay conditions were optimal for the different lysosomal hydrolases of different cell types and were linear with time and protein concentration up to 0.5 mg.

Protein was determined by the method of Lowry et al. (22) using bovine serum albumin as standard. The specific activities are expressed as nmol per min per mg protein.

RESULTS AND DISCUSSION

Phase contrast micrographs (**Figs. 1–3**) of the three cell fractions show that the neuronal fraction contains perikarya with few processes, whereas the astroglial cells have retained most of their arborized processes. The oligodendroglial cells are round cells with few processes as reported by Fewster, Blackstone, and Ihrig (8) and Poduslo and Norton (23). The purity of each cell fraction was judged to be about 85–90% by phase microscopy. Moreover, the use of enzyme markers (choline acetyltransferase, dopamine-β-hydroxylase, UDP galactose ceramide galactosyl transferase and 2′,3′-cyclic nucleotide-3′-phosphohydrolase) indicated that contamination of the neuronal fraction by glial elements and contamination of both glial fractions by neuronal membranes were below 20%.

The data on the distribution of DNA and lysosomal hydrolase activities in neuronal, astroglial, and oligodendroglial fractions of rabbit and beef brain are

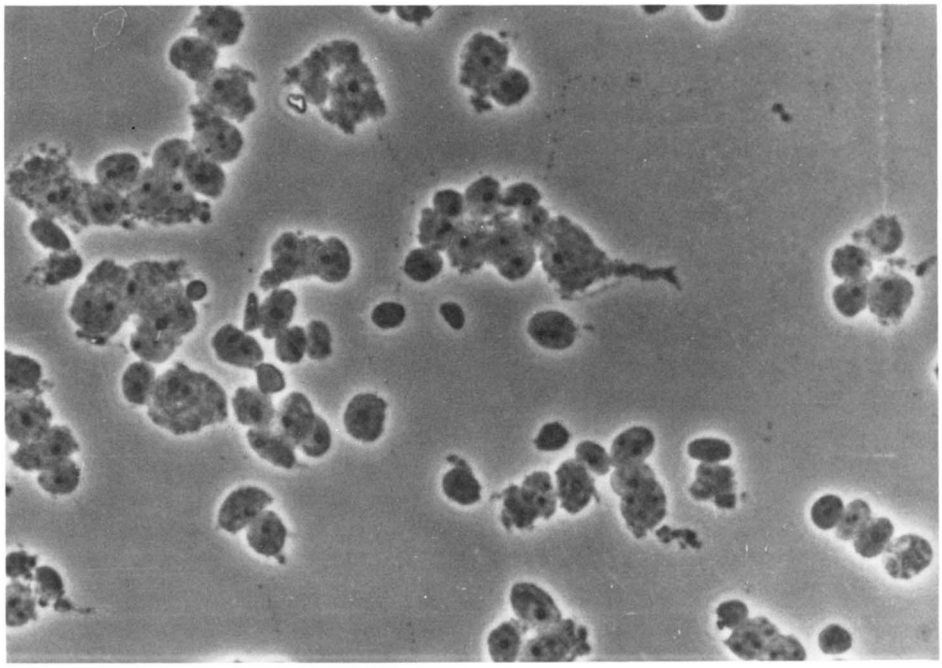

Fig. 1. Phase contrast photomicrograph of the neuronal fraction of brain cells isolated from rabbit brain. 400×.

presented in **Tables 1** and **2.** It is interesting to note that oligodendrocytes have more DNA per mg protein than neurons. A similar observation was also made by Fewster et al. (8) and Poduslo and Norton (23) for bovine and calf brains. The specific activities of all acid hydrolases are lower in beef brain cells than in rabbit brain cells. In both animal species the gray and white matter show similar specific activities for all lysosomal enzymes studied. However, it is obvious that the neuronal fraction of both beef and rabbit contains the highest activity of all lysosomal hydrolases. In rabbit brain, the oligodendroglial cell frac-

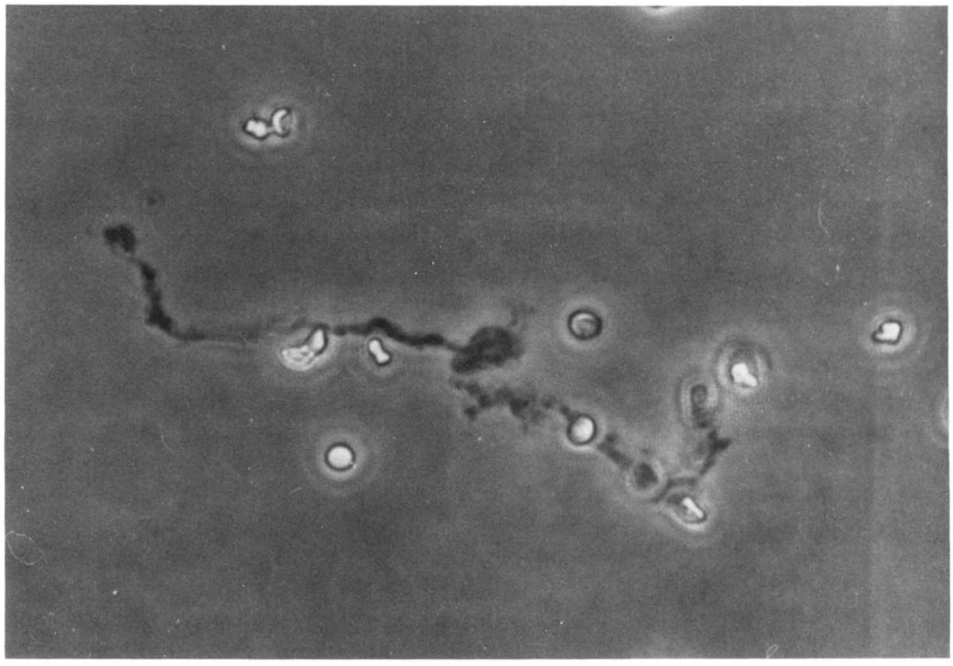

Fig. 2. Phase contrast photomicrograph of the astroglial fraction of brain cells isolated from rabbit brain. 400×.

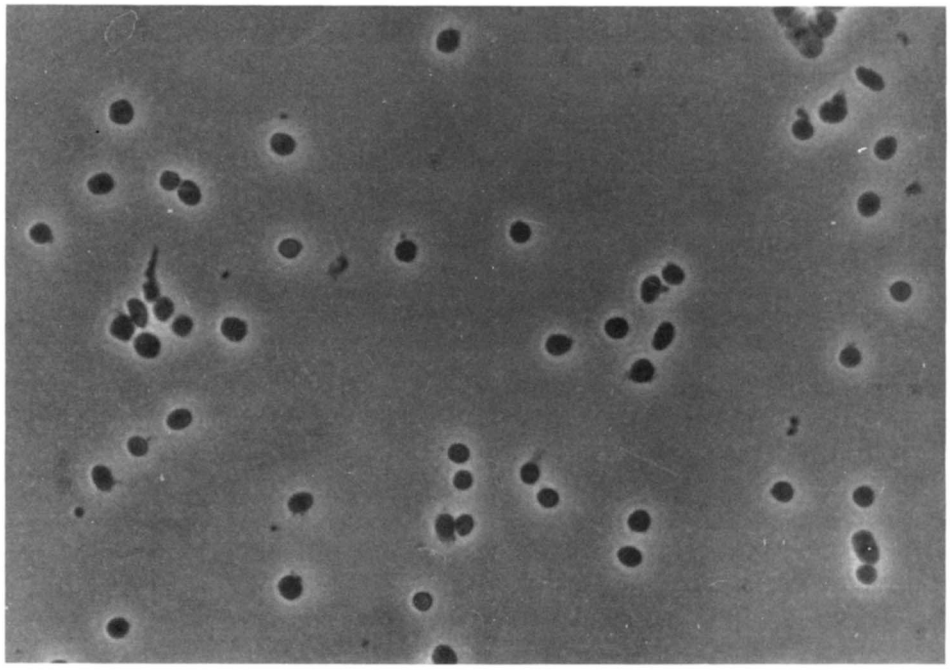

Fig. 3. Phase contrast photomicrograph of the oligodendroglial fraction of brain cells isolated from rabbit brain. 400×.

tion showed the lowest activity of these hydrolases, whereas in beef brain the activities of lysosomal enzymes were similar in both glial fractions and several times lower than in neurons. When the results are expressed per unit DNA, which permits the comparison of the enzyme activities per cell, the data also indicate that neurons contain higher lysosomal hydrolase activities than astrocytes and oligodendrocytes. These observations are comparable with most of the cytological and cytochemical studies which indicate that dense body lysosomes are limited largely to the neuronal perikaryon (3). Histological studies have also revealed that there are only a few lysosomes in astroglia and oligodendroglia of rat brain (3). Furthermore in an earlier work (24) it was suggested from indirect evidence that in brain most of the arylsulfatase A activity was localized in cells other than oligodendroglia. The present study gives direct evidence for this suggestion and indicates that a greater proportion of lysosomal hydrolases is localized in the neuronal fraction of rabbit and beef brain than in other fractions. Sinha and Rose (16) also reported that the lysosomal enzymes were concentrated more in the neuronal fraction than in the neuropil fraction of the adult rat brain. They suggested that β-galactosidase activity may in fact be a marker enzyme for the neuronal fraction. Our results on β-galactosidase activity are in agreement with those of Sinha and Rose (16). We found that in rabbit brain the activities of β-galactosidase were about 20 times higher in neuronal fraction than in oligodendroglial and astroglial cell fractions. The results on the activity of acid phosphatase agree very well with the histochemical observations of Hirsch (25, 26) who reported a relative abundance of acid phosphatase in neuronal perikarya compared to axons, dendrites, and glial cells. In contrast to Sinha

TABLE 1. Specific activities of acid hydrolases and DNA content in neuronal, astroglial, and oligodendroglial cells isolated from rabbit brain

Enzymes	Gray Matter	White Matter	Neuronal Cells	Astroglial Cells	Oligodendroglial Cells
Arylsulfatase A	2.85 ± 0.2	2.49 ± 0.1	3.66 ± 0.2	0.67 ± 0.03	0.25 ± 0.005
Arylsulfatase B	2.95 ± 0.1	4.73 ± 0.3	5.19 ± 0.3	0.90 ± 0.05	0.57 ± 0.007
Arylsulfatase C	0.078 ± 0.03	0.073 ± 0.005	0.31 ± 0.02	0.069 ± 0.001	0.28 ± 0.009
β-Galactosidase	1.11 ± 0.05	1.17 ± 0.07	2.25 ± 0.1	0.10 ± 0.001	0.12 ± 0.0005
Acid phosphatase	20.1 ± 1.5	14.4 ± 0.8	10.9 ± 1.6	0.92 ± 0.3	0.39 ± 0.0006
DNA μg/mg protein	3.33 ± 0.07	3.85 ± 0.04	47.4 ± 2.3	28.1 ± 1.2	69.2 ± 2.9

Values are expressed as nmol product formed per mg protein per min. Each value is a mean of five experiments ± SD.

TABLE 2. Specific activities of acid hydrolases and DNA content in neuronal, astroglial, and oligodendroglial cells isolated from beef brain

Enzymes	Gray Matter	White Matter	Neuronal Cells	Astroglial Cells	Oligodendroglial Cells
Arylsulfatase A	0.43 ± 0.01	0.35 ± 0.02	0.80 ± 0.03	0.16 ± 0.001	0.12 ± 0.002
Arylsulfatase B	0.82 ± 0.03	0.29 ± 0.01	1.34 ± 0.08	0.18 ± 0.0005	0.14 ± 0.001
Arylsulfatase C	1.30 ± 0.1	0.34 ± 0.03	2.65 ± 0.3	0.11 ± 0.0003	0.18 ± 0.006
β Galactosidase	0.25 ± 0.01	0.30 ± 0.005	0.35 ± 0.003	0.06 ± 0.0002	0.081 ± 0.003
Acid phosphatase	0.40 ± 0.02	0.25 ± 0.004	0.6 ± 0.06	0.07 ± 0.0003	0.12 ± 0.001
DNA μg/mg protein	1.00 ± 0.09	4.08 ± 0.02	41.2 ± 2.5	37.2 ± 0.4	155.0 ± 2.9

Values are expressed as nmol product formed per mg protein per min. Each value is a mean of five experiments ± SD.

and Rose (16), Hirsch (25, 26), and our results, Raghaven et al. (17) could not find any significant differences in the distribution of arylsulfatase, β-galactosidase, and acid phosphatase in neurons and astrocytes from young rat brain. This discrepancy can be explained by the age differences of the animals. Arbogast and Arsenis (27) observed that the specific activities of several lysosomal enzymes varied considerably in different cells of rat brain during development and that at the time of myelination the specific activities of these enzymes were similar in neurons and in glial cells.

The activities of microsomal arylsulfatase C (20) were also several times higher in beef neurons than in astroglial cells and oligodendroglial cells. However, in rabbit brain, activity of this enzyme is similar in neurons and oligodendroglia and about 4.5 times lower in astrocytes.

The specific activities of the various acid hydrolases are of the same order of magnitude in gray matter, white matter, and neurons, and are much higher than in both glial cells. Since white matter does not contain any neuronal perikarya but only axons and nonneuronal cells (mainly oligodendroglial and astroglial cells) (28), it should be expected that the contribution of the oligodendroglial cell hydrolase activities to the total white matter may be very low. It was reported by Nurnberger (28) that white matter contains about 7.8×10^7 cells per g fresh weight. Thus if we assume that all these cells are oligodendrocytes, the contribution of these cells toward the hydrolase activities of white matter is about 0.70% for rabbit and about 1.6% for beef.

Cerebrosides and sulfatides are mainly located in myelin sheath (29–32). Neurons and glial cells contain small amounts of these lipids (33–37). Since myelin is formed by the oligodendroglial cells, it can be expected that these cells will have the highest capacity for synthesizing these lipids. Indeed, Benjamins et al. (38) reported that the oligodendroglial cell fraction of calf brain had a higher 3′-phosphoadenosine-5′-phosphosulfate (PAPS) cerebroside sulfotransferase activity than neurons. Similar results were also obtained for uridine diphosphate (UDP) galactose ceramide galactosyl transferase of rabbit brain.[4] The low activities of arylsulfatase A and β-galactosidase in astroglial and oligodendroglial cells may suggest that the turnover of sulfatides and cerebrosides may be slower in these cells than in neurons, and that these enzymes may play some role in the control of the metabolism of these lipids. This suggestion is consistent with the observations of Davison and Gregson (30) and Jungalwala (31) who reported a very low turnover of sulfatide in adult rat brain compared to young animals, and with those of Arbogast and Arsenis (27) who observed that during myelination the activities of arylsulfatase A and other lysosomal enzymes are similar in neurons and astroglial cells.

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