CEREBRAL LYSOSOMES. VI. THE *IN VIVO* UPTAKE OF TRITON-WR-1339 BY THE LYSOSOMES OF THE IMMATURE CEREBRAL CORTEX AND CEREBELLUM

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INTRODUCTION

A number of recent reviews on neural lysosomes[6,8-10] have stressed the difficulties intrinsic to the isolation of these organelles in pure form from homogenates of brain tissue. We believe that this is a direct reflection of the remarkable lysosomal heterogeneity[13] of brain, which, in turn, derives from the complex mosaic of functional specificities exhibited by this organ. In two recent publications, we described the preparation of partially purified lysosomal fractions from several neuroanatomical areas of the rat brain[16] and presented comparative data relating the *in vitro* stability of lysosomes isolated from immature and adult brain cortex, thalamus and hypothalamus toward certain ionic and osmotic factors[17].

In this paper we describe the *in vivo* uptake of Triton-WR-1339 (ref. 20) by the lysosomes of the brain cortex and the cerebellum of the immature rat and the effects of this functional event on their centrifugal properties. A preliminary account of some of the findings has appeared[18].

EXPERIMENTAL

Materials

Chemicals

o-Nitrocatechol, *o*-nitrocatechol sulfate (K salt), acetylthiocholine iodide, 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride (INT): Sigma Co., St. Louis, Mo.; *o*- and *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide: Pierce Chemical Co., Rockford, Ill.; 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB): K and K Labs., Plainview, N.Y.; Triton-WR-1339 (polyethylene glycol derivative of polymerized *p-tert.*-octylphenol): Rueger Chemical Co., Irvington-on-Hudson, N.Y.; materials for electron microscopy: Ladd Research Industries Co., Burlington, Vt.

Animals

Male Sprague–Dawley rats, 12–13 days old, were used.

Methods

Analytical

Protein was determined according to Lowry *et al.*[11] with bovine serum albumin as standard.

Enzymic

Arylsulfatase (EC 3.1.6.1) and *N*-acetyl-β-D-glucosaminidase (EC 3.2.1.30) (NPG-ase) were measured as previously described[16]. For simplicity, the activity of the enzymes is expressed as U./g, defined as absorbancy (at 515 nm for sulfatase and at 412 nm for NPG-ase)/h/g. Acetylcholinesterase (EC 3.1.1.7) was measured according to Ellman *et al.*[3] and succinate-INT-reductase according to Hunter and Millson[7]. Glutamine synthetase (EC 6.3.1.2) was assayed according to Sellinger *et al.*[15].

Centrifugal fractionation of the cerebral cortex and cerebellum

The cerebral cortex and the cerebellum were dissected, placed in ice-cold 0.25 *M* sucrose and their weight determined. Centrifugation of the homogenate proceeded as described in detail in our previous publication[15]. Briefly, the 10–15% (w/v) homogenate was centrifuged for 10 min at 25,000 $\times$ *g*, the sediment resuspended in half the original volume of 0.25 *M* sucrose and the above centrifugation repeated. The pellet was suspended in 0.25 *M* sucrose and layered on top of 15 ml of 0.9 *M* sucrose underlayered by 10 ml of 1.3 *M* sucrose. Centrifugation at 63,000 $\times$ *g* for 30 min separated myelin (fraction MY) floating on the 0.9 *M* sucrose, from a particulate fraction recovered on top of the 1.3 *M* sucrose (fraction NEM) and which consisted of nerve endings, mitochondria and some lysosomes. The material traversing the 1.3 *M* layer and forming a pellet is designated as the crude lysosomal fraction (LY). For separation of the microsomal fraction (MIC), the supernatant remaining after the second 25,000 $\times$ *g* step (see above) was centrifuged for 45 min at 269,000 $\times$ *g*. The resulting supernatant is designated fraction S. In some experiments, the homogenate was initially centrifuged for 10 min at 1,000 $\times$ *g* and washed for 8 min at the same speed to sediment the nuclear fraction (N). For additional purification, fraction LY was subjected to centrifugation in a linear gradient of 15–45% (w/v) of sucrose for 2.5 h at 63,500 $\times$ *g*. The gradient was pumped through the bottom of the polyallomer centrifuge tube by means of a proportioning pump (Technicon, Inc., Chauncey, New York) into test tubes resting in fraction collector racks. The effluent was collected at a rate of 10 drops/tube. The pellet is designated as the fraction of purified lysosomes (fraction PLY).

Electron microscopy

Samples of fraction PLY (see above) in pellet form were fixed for 2 h in ice-cold 1.5% glutaraldehyde in 0.1 *M* phosphate buffer (pH 7.4) and were post-fixed in a 2% (w/v) solution of ice-cold OsO_4 in 0.1 *M* phosphate buffer, pH 7.4, also containing 5% (w/v) sucrose for an additional 2 h. After a brief wash in the phosphate buffer, the

TABLE I

EFFECT OF pH AND TEMPERATURE ON THE STABILITY OF CEREBRAL LYSOSOMES

Fraction LY was prepared from the cerebral cortex as outlined in Methods and was suspended in the various media as indicated. The concentration of all buffers was 0.015 *M*. Incubation was for 1 h at the temperature indicated. The suspensions were directly loaded on 15–45% (w/v) linear gradients of sucrose and were centrifuged for 2.5 h at 63,500 × *g*. Values refer to the percentages of the layered activities which were recovered in the pellet (fraction PLY).

Lysosomal hydrolase	*Suspension medium*									
	0.25 M sucrose		*Acetate (pH 5.1)*		*Acetate (pH 5.1) + 0.25 M sucrose*		*Imidazole (pH 6.4)*		*Tris (pH 7.2)*	*Tris (pH 7.2) + 0.25 M sucrose*
	0°C	*37°C*	*0°C*	*37°C*	*0°C*	*37°C*	*0°C*	*37°C*	*0°C*	*0°C*
Arylsulfatase	44	24	29	22	33	19	20	17	16	32
NPG-ase*	59	53	48	40	38	33	40	32	72	48

* *N*-acetyl-β-D-glucosaminidase.

fixed material was dehydrated at room temperature in a graded alcohol series. The procedure for whole tissue consisted of fixation of the finely minced specimen in 3% buffered glutaraldehyde, as above, followed by post-fixation in 1% OsO_4. All fixed specimens were washed 3 times with pure propylene oxide, once with propylene oxide–Epon 812 (1:1, v/v) and once with pure Epon 812. Embedding was in Epon 812 of medium hardness in 'beem' capsules with successive 24 h polymerizations at 37°, 45° and 60°C. The ultra-thin sections were mounted on bare 400-mesh copper grids and were stained with 5% (w/v) uranyl acetate in 50% aqueous ethanol. They were counterstained for no longer than 15 min with lead citrate and examined in a JEOLCO, JEM 6A electron microscope.

Injection of Triton-WR-1339

Nine to 11-day-old animals were lightly anesthetized by exposure to ether vapors and 50 μl of 20% (w/v) Triton-WR-1339 in 0.15 *M* NaCl were injected intrathecally. Injections were repeated under identical conditions at intervals of 24 h. No gross behavioral or toxic effects were noted.

RESULTS

Search of optimal conditions for the purification of fraction PLY

The procedural step whereby a relatively impure preparation of lysosomes (fraction LY, see Methods) is centrifuged on a linear density gradient of sucrose to yield the somewhat purer fraction PLY as a pellet has been used in the past as a convenient test system for studies of the effect of a number of agents and conditions on *in vitro* lysosomal stability[17]. As shown by the results of Table I, incubation of fraction

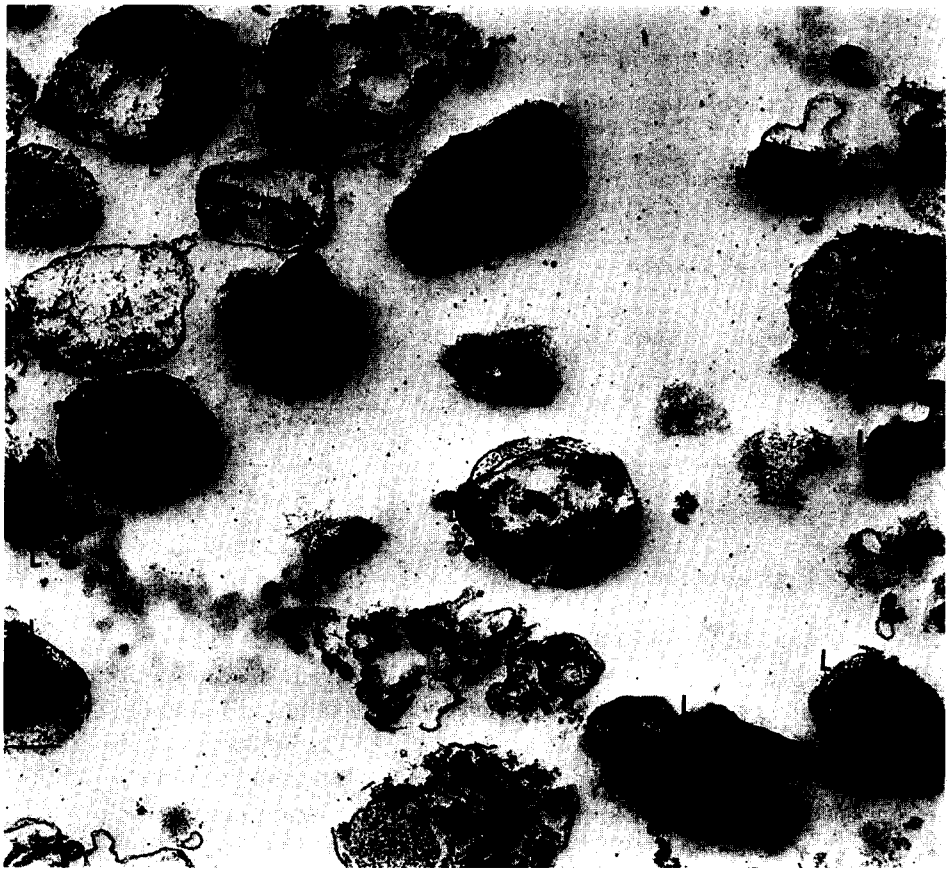

Fig. 1. Electron micrograph of fraction PLY. The fraction was isolated from the cerebral cortex of 13-day-old rats by the centrifugal procedures described in Methods. L, lysosomes or cytolysosomes; M, mitochondria or their identifiable remnants. Note the presence of 12 lysosomes of varying sizes and shapes and, presumably, functional states (cytolysosomes). Also note the absence of identifiable nerve endings containing synaptic vesicles or of synaptic vesicles themselves. Three mitochondria or mitochondrial remnants are also present. × 48,000.

LY in media of increasing pH led to a decrease in the recovery of arylsulfatase in fraction PLY, but not of that of NPG-ase. Similarly, incubation in 0.25 *M* sucrose alone or in isotonic acetate or Tris buffers led to some solubilization of arylsulfatase activity but in negligible losses of sedimentable NPG-ase activity. It could also be shown that both hydrolases were more sensitive to pH at 37°C than at 0°C. Suspension in 0.25 *M* sucrose at 0°C was selected for the routine preparation of fraction PLY.

Relative purity of fraction PLY

Although the representative field covered by the electron micrograph of fraction PLY (Fig. 1) contains 3 identifiable mitochondria and/or mitochondrial remnants and several ill-defined membrane fragments, it also contains 12 bodies identified as lyso-

TABLE II

THE EFFECT OF TRITON-WR-1339 ON LYSOSOMAL YIELD

Rats were injected intrathecally with Triton-WR-1339 as described in Methods. They were killed on their 12th or 13th day of life and the cerebral cortex and cerebellum immediately homogenized in 0.25 *M* sucrose. Fraction LY was prepared as described in Methods. The total activity of arylsulfatase and of NPG-ase in the homogenates was determined and taken to represent 100%. The yield in fraction LY is expressed as the percent of the total activity actually found in the fraction, without correction for differences in recovery. These could not be ascertained with precision since the method used to prepare fraction LY involves centrifugation through discontinuous gradients and the large volumes of sucrose separating the regions where particulate material collects are discarded. The values are means from 3 experiments with CC and averages from 2 experiments with CL.
CC, Cerebral cortex; CL, cerebellum; NPG-ase, *N*-acetyl-β-D-glucosaminidase.

Lysosomal hydrolase	*No Triton*				*Triton (2 days)*				*Triton (3 days)*			
	U./g*		*Yield (%)*		*U./g*		*Yield (%)*		*U./g*		*Yield (%)*	
	CC	*CL*	*CC*	*CL*	*CC*	*CL*	*CC*	*CL*	*CC*	*CL*	*CC*	*CL*
Arylsulfatase												
Total	145	85.2	100		129	94.5	100		88.5	96.5	100	
Fraction LY	68	37.4	46.8	43.7	18.7	31.7	14.4	33.3	10.0	18.8	11.3	19.0
NPG-ase												
Total	44.6	27.1	100		48.5	29.5	100		45.0	33.3	100	
Fraction LY	16.7	8.1	37.6	29.8	6.55	5.95	13.5	20.2	6.9	4.4	15.7	13.3

* Unit: absorbancy/h (see Methods).

somes or functionally similar particles, such as cytolysosomes. The enzymic analysis of the fraction denoted little contamination by marker-enzymes of other membranous components, namely succinate-INT-reductase, acetylcholinesterase and glutamine synthetase which reflect, respectively, the presence of mitochondrial and of synaptic and microsomal membranes[15,17].

The effect of Triton-WR-1339

(A) On the activity of the hydrolases. When the total activities of arylsulfatase and NPG-ase were determined at intervals after initiating the administration of Triton-WR-1339, a decrease of the cortical arylsulfatase but not of the cerebellar enzyme was noted over the 3-day period of treatment (Table II). Triton-WR-1339 had no effect on the NPG-ase of either neuroanatomical area.

(B) On the yield of protein and of the hydrolases in fraction LY. The percentage of the total cortical arylsulfatase recovered in fraction LY decreased from 46.8 in controls to 11.3 after 3 days of Triton-WR-1339. The comparable values for NPG-ase were 37.6 and 15.7% (Table II). The protein content of fraction LY was only slightly diminished. Consequently, the relative specific activity (percent of total activity/percent of total protein) (RSA) of the cortical arylsulfatase in fraction LY dropped

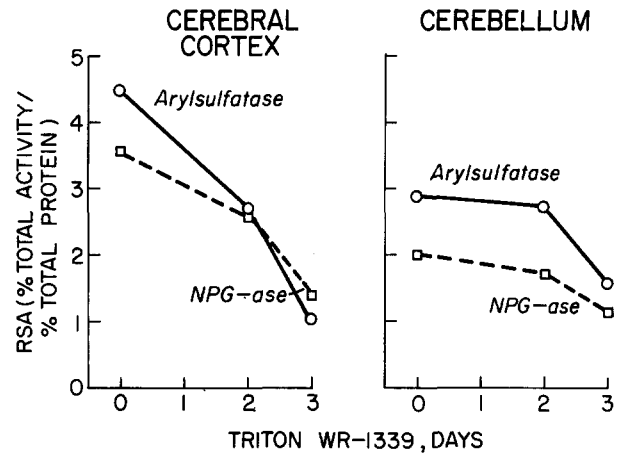

Fig. 2. The relative specific activity (% of total activity/% of total protein) (RSA) of cortical and cerebellar arylsulfatase and NPG-ase as a function of the *in vivo* administration of Triton-WR-1339. The RSA values were determined in fraction LY of control animals and of animals injected twice or three times with Triton-WR-1339 as described in Methods. Each point is the mean of at least 3 experiments.

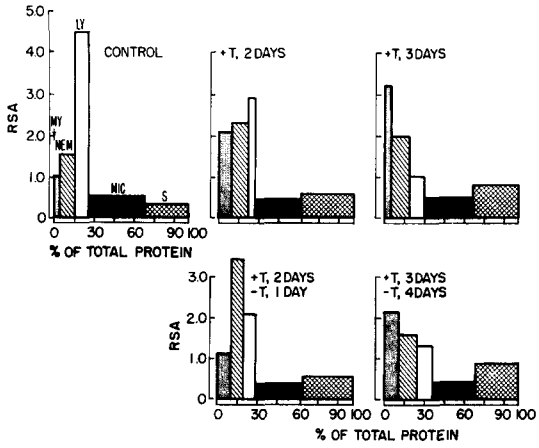

Fig. 3. Effect of the *in vivo* administration of Triton-WR-1339 on the intracellular distribution of arylsulfatase in the cerebral cortex. The subcellular fractions MY, NEM, LY, MIC and S, defined operationally in the text (Methods), were isolated from control animals and from animals injected with Triton-WR-1339, as indicated. T = Triton-WR-1339.

after 3 days of treatment from 4.5 to 1.0 and that of NPG-ase from 3.6 to 1.4 (Fig. 2). A similar situation prevailed in the cerebellum, the percentage of arylsulfatase in fraction LY decreasing from 43.7 to 19.0 and that of NPG-ase from 29.8 to 13.3. Fig. 2 illustrates the downward trend of the RSA values for the two enzymes, a visibly slower process in the cerebellum.

(C) On the centrifugal 'shift' of the lysosomes. The lysosomal location of arylsulfatase and NPG-ase in both the immature and the adult rat brain cortex has been

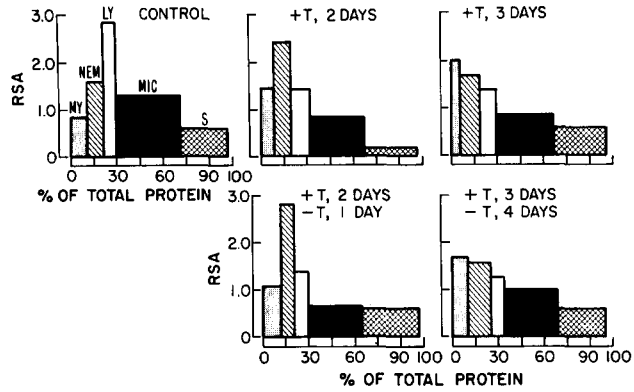

Fig. 4. Effect of the *in vivo* administration of Triton-WR-1339 on the intracellular distribution of NPG-ase in the cerebral cortex. For details, see the legend of Fig. 3. T = Triton-WR-1339.

TABLE III

THE EFFECT OF TRITON-WR-1339 ON THE SPECIFIC ACTIVITY* OF ARYLSULFATASE AND NPG-ASE IN CEREBELLAR SUBCELLULAR FRACTIONS

Cerebella of control and Triton-injected rats were homogenized in 0.25 *M* sucrose and the homogenates subjected to centrifugation to isolate the fractions as indicated. The details of the procedure and the operational definition of all the fractions is provided in the section Methods.

Subcellular fraction	*No Triton*		*3 days of Triton*	
	*Arylsulfatase (3)***	*NPG-ase (3)*	*Arylsulfatase (2)*	*NPG-ase (2)*
Myelin	2.54	0.58	3.20	1.24
Nerve endings + mitochondria	4.74	1.09	3.68	1.09
Lysosomal	4.15	0.95	2.35	0.88
Microsomal	1.06	0.62	0.71	0.72
Soluble	0.48	0.18	0.76	0.30

* U./mg of protein; NPG-ase: *N*-acetyl-β-D-glucosaminidase.
** Indicates number of experiments.

fully documented previously[13,15]. Mordoh[12] and, more recently, Clendennon and Allen[2] have studied the intracellular localization of arylsulfatase and of other acid hydrolases in brain, but neither group has examined the influence of *in vivo* administered agents on these enzymes. Several years ago, we reported on the *in vivo* 'labilization' of cerebral lysosomes by the convulsant agent methionine sulfoximine[19]. Figs. 3 and 4 illustrate the effect of the different schedules of Triton-WR-1339 administration on the intracellular distribution of the hydrolases in the cortex. The histograms show a shift from fraction LY to lighter fractions which was already noticeable after 2 days of treatment but which became very pronounced after 3 days, at which time the RSA values of both enzymes were highest in the fraction containing myelin

Fig. 5. Electron micrograph of the cerebral cortex of a rat after 3 days of intrathecal administration of Triton-WR-1339. Triton-WR-1339 was injected intrathecally beginning on day 9 and continued daily on days 10 and 11. The animals were killed on day 12 and the cortical tissue was processed for electron microscopy as outlined in Methods. TFL, Triton-filled lysosome; M, mitochondrion; GA, Golgi apparatus; FR, free ribosomes (not attached to the membranes of the endoplasmic reticulum); BR, ribosomes attached to the membranes of the endoplasmic reticulum; NM, nuclear membrane; N, nucleus; NL, nucleolus; SER, smooth endoplasmic reticulum. × 40,000.

and the 'light' nerve endings (fraction NEM). It should also be noted that after 3 days of Triton the RSA of NPG-ase in fraction LY remained above unity while that of the more soluble arylsulfatase decreased to values below unity. Concomitantly, while the RSA of arylsulfatase in fraction S increased that of NPG-ase did not. The histograms further show that interrupting the administration of Triton-WR-1339 for up to 4 days did not cause the return of the lysosomes to control sedimentation patterns. The results shown in Table III confirm that the peak of the cerebellar arylsulfatase also shifted from fraction LY to fraction MY as a consequence of the Triton treatment. Yet, this process was much slower in this brain area than in the cortex, as evidenced by a value of 2.32 (down from 4.15 in controls) for the specific activity of the enzyme in fraction LY after 3 days of Triton-WR-1339 as well as by the minimal rise

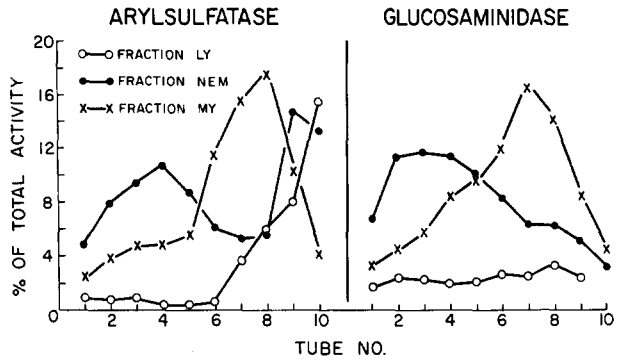

Fig. 6. A comparison of the sedimentation rates of lysosomes contained in different subcellular fractions of Triton-treated cerebral cortices. Triton-WR-1339 was administered intrathecally for 3 days and on day 4 fractions MY, NEM and LY (see Methods for their operational definition) were isolated. Suspensions of these fractions were layered on identical, linear 15–45% sucrose gradients and the tubes were centrifuged at 63,500 × *g* for 2.5 h. Arylsulfatase and NPG-ase activities were determined in the collected effluent (see Methods), and as noted in the text, in the pellets as well. No pellet was obtained from fraction MY. Arylsulfatase activity was high on top of the gradients obtained from fractions NEM and LY, denoting leakage of this hydrolase from the lysosomes during the gradient run. Conversely, NPG-ase was not rendered non-sedimentable under these conditions. Also note that the peaks of arylsulfatase (tube 8) and NPG-ase (tube 7) in the gradient obtained from fraction MY were well inside the sucrose, suggesting a true association of these hydrolases with the newly formed myelin.

in specific activity of the enzyme in fraction MY (from 2.54 to 3.20). As in the cortex, Triton-WR-1339 elicited some solubilization of arylsulfatase, as shown by the increase of its specific activity in fraction S (from 0.48 to 0.76). The movement of NPG-ase (Table III) was a further confirmation of the mild effects elicited by Triton-WR-1339 in the cerebellum. These consisted mainly in a doubling of its specific activity in fraction MY (from 0.58 to 1.24), a slight drop of its specific activity in fraction LY (from 0.95 to 0.88), no change in fraction NEM and, lastly, in a small but consistent increase of its specific activity in the microsomal fraction.

Characterization of the Triton-filled lysosomes

As shown above, a large portion of the cortical lysosomes sedimented in fraction NEM rather than in fraction LY after 3 days of Triton administration. Presumably this occurred because, as they filled with the detergent, lysosomes became lighter[20] (Figs. 3 and 4). The electron micrograph of the brain cortex of a treated rat (Fig. 5) shows several Triton-filled lysosomes whose appearance strongly resembles their hepatic counterparts[20]. The micrograph also illustrates the absence of other detectable ultrastructural changes resulting from the intake of the detergent. To demonstrate that the Triton-filled lysosomes constitute a distinct population of granules with sedimentation characteristics of their own, they were compared to lysosomes which normally sediment in fraction NEM of untreated animals by virtue of their being lighter than those sedimenting in fraction LY (see control histograms of Figs. 3 and 4). These experiments revealed that in contrast to the control fraction NEM, fraction NEM of

the Triton-treated cortices actually contained a population of granules which failed to sediment to the bottom of the 15–45% sucrose gradient (see Methods). This was true even after 3.5 h of centrifugation. Assay of the gradient pellet corroborated these findings by revealing a considerably larger proportion of hydrolase activity in the pellets derived from control fraction NEM than in those derived from the fraction NEM of Triton-treated animals. When fraction MY of the Triton-treated animals was centrifuged through the 15–45% sucrose gradient, both hydrolases showed activity peaks near the gradient top (Fig. 6), there being no pellet.

Additional inspection of Figs. 3 and 4 shows that even after 3 days of Triton-WR-1339 administration, some lysosomes remained in fraction LY and hence contained little or no Triton-WR-1339. Fig. 6 shows that when these lysosomes were sedimented through the 15–45% sucrose gradient, 63.3% of the arylsulfatase and 78.8% of the NPG-ase were recovered in the pellet.

Further experiments were carried out to assess whether Triton-WR-1339 was taken up by any other intracellular organelles. An experiment in which the intracellular distributions of succinate-INT-reductase, acetylcholinesterase and glutamine synthetase were examined in cortices of animals which received Triton-WR-1339 for 3 days revealed distribution patterns which were indistinguishable from companion control patterns.

DISCUSSION

The results of Table I show that arylsulfatase and NPG-ase were maximally recovered in fraction PLY after resuspension of fraction LY in an ion-free medium of 0.25 *M* sucrose at 0°C. Of interest was the coincidental finding that the arylsulfatase was more susceptible to leakage from lysosomes than was the more structure-bound NPG-ase[16,17]. This was particularly evident after resuspension of fraction LY in Tris, pH 7.2, or imidazole, pH 6.4, buffers. In this respect arylsulfatase resembles cerebral β-glucuronidase[9].

In his recent reviews[8–10] Koenig has described the uptake of the intravital dye neutral red by the lysosomes of the brain. However, as noted by this author, the intraperitoneal administration of the dye in a dose range of 335–400 mg/kg caused 'remarkable changes' in lysosomal structure. Also, Koenig failed to detect any neutral red in lysosomes 18 h after its administration[9].

In the present study the injection of Triton-WR-1339 by the intrathecal route resulted in the uptake of this detergent by the lysosomes of the cerebral cortex as well as, albeit less efficiently, by those of the cerebellum. Wattiaux *et al.*[20] noted a rather slow uptake of Triton-WR-1339 by liver lysosomes over a period of 3 days, at the end of which time practically the entire hepatic lysosomal population had become filled with it. To obtain a sustained uptake by the lysosomes of the cerebral cortex, we found it necessary to administer Triton-WR-1339 repeatedly, rather than in a single dose. As may be expected, uptake by the lysosomes of the cerebellum was delayed *vis-à-vis* the cortical uptake, presumably because of the greater proximity of the latter area to the injection site (Fig. 3).

In their original report on the uptake of Triton-WR-1339 by liver lysosomes, Wattiaux *et al.*[20] reported no effect of the detergent on the activity levels of acid phosphatase, the only lysosomal hydrolase tested by these authors. Our results show a selective effect of the detergent on the cortical arylsulfatase whose activity decreased by about 40% after 3 days of treatment (Table II). The injection of Triton-WR-1339 also caused an apparently nonselective leakage of the cortical and cerebellar arylsulfatase from lysosomes as evidenced by its rather high RSA values in fraction S (Fig. 3 and Table III). A similar 'hydrolase escape' from liver lysosomes has been noted by Weissman and Uhr[21] following the injection of Thorotrast. The histograms of Figs. 3 and 4 show that, once filled with Triton-WR-1339, lysosomes do not readily give up the detergent. Thus, the intracellular distribution of the hydrolases varied very little between the two conditions of 3 days of Triton treatment and death on day 4, and 3 days of Triton treatment, 4 days of no treatment and death on day 8. Wattiaux *et al.*[20] thought it 'remarkable' that practically all liver lysosomes became filled with Triton-WR-1339 after a single intravenous injection. In our experience, in which Triton-WR-1339 was injected intrathecally daily for 3 days, it was always possible to isolate some lysosomes, particularly from fraction NEM (Fig. 6), which penetrated the entire linear sucrose gradient routinely used for the preparation of fraction PLY (Table I). Furthermore, since the percentages of arylsulfatase and NPG-ase contained in fractions LY of the cortex and of the cerebellum of Triton-treated animals were rather similar (11.3 and 19%, Table II), it appears that in both brain areas a rather constant proportion of lysosomes was refractory to Triton-WR-1339. If it is true that in brain, as in the other systems tested, Triton-WR-1339 enters only 'secondary' lysosomes[4,20], the present findings mean that about 80–85% of the total population of lysosomes are of this type in the immature brain cortex, the rest presumably being represented by primary, pre-functional types. Fig. 1 lends additional support to this possibility.

The finding that lysosomes of the immature brain cortex and cerebellum efficiently take up intrathecally administered Triton-WR-1339 is compatible with the ideas which argue in favor of a universality of lysosomal function across normal cell types. Lysosomes of immature brain cells would thus perform digestive tasks as readily as their extracerebral (mature) counterparts, provided, of course, that the actual physiological occurrence of a digestive event may be surmised on the basis of the experimentally demonstrated ability of lysosomes to take up exogenous Triton-WR-1339. Related observations in support of the role of cerebral lysosomes in processes of exocytosis are the capture of horseradish peroxidase by neurons and neuroglia following the implantation of this plant protein in the cerebrum[1], the uptake of this enzyme by cultured neurons of the dorsal root ganglion[5] and, more directly, by the selective uptake of intrathecally administered horseradish peroxidase by neurons of the immature rat brain cortex, recently observed in our Laboratory[14].

SUMMARY

The detergent Triton-WR-1339 was injected intrathecally into immature rats and its uptake by the lysosomes of the cerebral cortex and the cerebellum was dem-

onstrated. Uptake was assessed *in vitro* by examining the shift of the lysosomes to centrifugal fractions sedimenting at lower speeds than those commonly yielding these granules from control brains. This shift was maximal after 3 daily injections of Triton when arylsulfatase and *N*-acetyl-β-D-glucosaminidase, the two lysosomal hydrolases studied, exhibited peaks of relative specific activity (RSA) in fractions known to contain myelin fragments and nerve endings rather than in the heavier fraction known to concentrate lysosomes. A significant proportion of the cortical and cerebellar lysosomes failed to take up the detergent even after 3 days of continued administration.

The apparent irreversibility of the uptake process was suggested by the finding that the RSA values for the two hydrolases were still highest in the myelin and light nerve ending fractions 4 days after the administration of Triton-WR-1339 had been discontinued. Sedimentation in linear density gradients of sucrose readily separated the populations of Triton-filled and Triton-devoid lysosomes.

The results demonstrate the ability of cerebral lysosomes to perform a functional task characteristic of their extracerebral counterparts. It has not been established whether the uptake of Triton-WR-1339 was selective into the lysosomes of neuronal or glial cells or whether it proceeded uniformly into both cell types.

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