Glycosidases in the Nervous System

I. ASSAY, SOME PROPERTIES, AND DISTRIBUTION OF β-GALACTOSIDASE, β-GLUCURONIDASE, AND β-GLUCOSIDASE*

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SUMMARY

An intensive study of glycosidases in nervous tissue was done. An existing method, utilizing 4-methylumbelliferyl-β-D-glycosides, was modified to permit the assay of a given glycosidase in as little as 1 μg of nervous tissue. The distribution of β-galactosidase, β-glucuronidase, and β-glucosidase was studied by this method in various discrete regions of the nervous system. The relative distributions of β-galactosidase and β-glucuronidase were the same in cerebellar layers, the granular layer having the highest activity. In contrast, the molecular layer exhibited the greatest activity for β-glucosidase. All three enzymes showed a maximal concentration in the 12,000 and 18,000 × g sediments in sucrose homogenates with only 5 to 10% of their activities in the microsomes and cell sap. β-Galactosidase and β-glucuronidase were 50% soluble in aqueous media, whereas β-glucosidase was less than 10% soluble. The two former enzymes were completely active after homogenization in water, whereas treatment with a detergent almost doubled the total activity of β-glucosidase. The pH optima were lower for β-galactosidase and β-glucuronidase (4.0 in monkey and 3.75 in rat for the former, and 3.3 to 3.75 in both species for the latter) than for β-glucosidase (5.4 in both species). At high inhibitor concentrations each aldonolactone inhibited its corresponding enzyme without affecting the other two, further indicating that in nervous tissue these are at least three separate and distinct enzymes.

EXPERIMENTAL PROCEDURE

Analytical Procedure

Principle—The principle of the method was that initially used for the assay of β-glucuronidase (11), in which MU-glucuronide* was the substrate and the liberated 4-methylumbelliferone was measured. 4-Methylumbelliferone is highly fluorescent in alkaline solution. The fluorescence of the 4-methylumbelliferone

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A preliminary report of some of this work has appeared (1).

biochemical concept (see References 2 and 3 for reviews); and (b) the unraveling of the role of glycosidases in glycolipid metabolism (4-7). The characteristics of these enzymes have been explored in depth especially by Levvy and Conchie (3). These enzymes have not been studied to a great extent in brain. The finding of greatly enriched amounts of each of the glycosidases measured in individual nerve cell bodies (8, 9) led to an intensive study of three glycosidases in tissues of the nervous system. The enzymes are β-galactosidase (β-n-galactoside galactohydrolase, EC 3.2.1.23), β-glucuronidase (β-D-glucuronic acid glucuronohydrolase, EC 3.2.1.31), and β-glucosidase (β-D-glucose glucohydrolase, EC 3.2.1.21).

The purposes of the present report are to present a modification of an existing method for measuring the activities of the glycosidases that is simple and convenient, and that permits the measurement of the enzyme activity in as little as 1 μg or somewhat less of neural tissue; to present some properties of the three enzymes with regard to solubility, inhibitors, and kinetic constants in whole central nervous system tissue; to suggest that the three β-glycosidases are distinct enzymes (see Paper III (10) of the present series for a more detailed description of this point and of the characteristics and kinetic properties of the purified enzymes); to describe the distribution of these enzymes in various discrete areas of brain, in various white matter fiber tracts, and the quantitative histochemical distribution in layers of the cerebellar cortex and its subjacent white matter; and to describe the subcellular distribution of these enzymes in the central nervous system, utilizing sucrose as a suspending medium.

The glycosidases have in recent years been important in the exploitation of two areas in biochemistry: (a) the lysosome as a...
ferone is reduced by 4000- to 5000-fold, and the exciting wave
length is shifted to shorter values when the hydroxyl group of the
4-methylumbelliferone is substituted by a sugar to form a
glycoside and the last traces of 4-methylumbelliferone are
removed.

Materials—Gifts of MU-glucuronide and MU-glucoside from
Professor R. T. Williams, and of MU-galactoside from Professor
Melvin Cohn had negligible fluorescence. In our laboratory,
MU-glucoside synthesized chemically (12) and MU-glucuronide
synthesized biologically (11) gave about the same fluorescence as
the material donated to us, and gave the same activating and
fluorescence peaks as the materials from Professor Williams.
By using highly purified β-galactosidase from Escherichia coli
it was possible to show that the MU-galactoside was free of
detectable free 4-methylumbelliferone and that no α-galactoside
was present. (Each of the three 4-methylumbelliferone-β-
glycosides is now available commercially from Pierce Chemical
Company, Rockford, Illinois. Except for MU-glucoside, which
worked well, the two other commercial products have not yet
been used in our laboratory.)

Procedure—The stock homogenates, 1:5, were prepared in
redistilled water at 2°, unless otherwise specified. Except in
certain exceptional instances, all homogenizations were made
in an all glass homogenizer by hand. When stock homogenates
were diluted further, 0.05% bovine serum albumin was used as
a diluent. All reagents were prepared in redistilled water and
stored at -20°. They were indefinitely stable; although the
4-methylumbelliflorone standard should be checked monthly or
so. 4-Methylumbelliferone was obtained from Fisher Scientific
Company, Pittsburgh, “highest purity” grade; p-gluco-1,4-
lactone (n-saccharo-1,4-lactone) from Calbiochem; n-glucono-1,4-
lactone (n-saccharo-1,4-lactone) from Calbiochem; n-glucaro-
so. 4-Methylumbelliferone was obtained from Fisher Scientific
Company, Pittsburgh, “highest purity” grade; n-glucaro-

The central nervous system tissue and buffer-substrate were
incubated in appropriate volumes, in appropriately sized tubes,
and for optimal times, depending on the amount of brain, the
tissue. The tubes were read in a Farrand fluorometer, model
A2, with the No. 5860 Corning filter in the primary, to isolate
the emitted fluorescence between
3387 in the secondary,
the mercury line at 365 rnp, and Corning filters Nos. 5543 and
3387 in the secondary,

The experimental procedure consisted of placing 25 µl of
buffer-substrate mixture into a fluorometer tube (10
× 75 mm, Corning No. 9820) in an ice bath, adding the requisite amount of
brain in a 1- to 5-µl aliquot, incubating at 37° for exactly 1 hour,
returning the tubes to the ice bath, and adding 1 ml of 0.02 M
2-amino-2-methyl-1-propanol-HCl buffer, pH 10.3 to 10.4, to
stop the reaction. The solution was then at optimal pH for
maximal fluorescence of the 4-methylumbelliflorone. Standards

| Table 1
| Optimal assay conditions, Km and Vmax for three central nervous system β-glycosidases
| Constituent, Km or Vmax (units) | β-Glucosidase | β-Glucuronidase | β-Glucosidase |
| Monkey | Rat | Monkey | Rat | Monkey | Rat |
| Substrate (mM) | 1.1 | 1.1 | 1.0 | 1.0 | 2.9 | 2.9 |
| Mg++ (mM) | 5.0 | 5.0 | 1.0 | 1.0 | 2.9 | 2.9 |
| Bovine serum albumin (g%) | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Sodium acetate buffer pH | 4.0 | 3.75 | 3.75 | 3.75 | 5.1 | 5.4 |
| Kmax (mM) | 0.108 | 0.152 | 0.058 | 0.058 | 0.0186 | 0.072 |
| Vmax (mM per mg of fresh weight per hour) | 9 | 14 | 1.5 | 3 | 5 | 1.5 |

**Notes:**
- *Macaca mulatta.*
- The substrate in each instance was the corresponding
4-methylumbelliferyl-α-n-glucoside. The buffer-substrate was
prepared fresh the day of use or the day before. If prepared the
day before, it was kept frozen.
- The Km for lactose in the rat was much higher, 14.3 mM; Vmax
for lactose in the rat = 5.
- Range of Vmax over a period of 6 years, with >30 measurements of each enzyme in each species under the conditions noted in the table.

All concentrations were final concentrations present during
incubation.

The MU-glucuronide was not quite as pure, or had
less intrinsic fluorescence, reducing the limit sensitivity to about
2-fold. The substrate in each instance was the corresponding
4-methylumbelliferyl-α-n-glucoside. The buffer-substrate was
prepared fresh the day of use or the day before. If prepared the
day before, it was kept frozen.

The central nervous system tissue and buffer-substrate were
incubated in appropriate volumes, in appropriately sized tubes,
and for optimal times, depending on the amount of brain, the
tissue. The tubes were read in a Farrand fluorometer, model
A2, with the No. 5860 Corning filter in the primary, to isolate the
mercury line at 365 rnp, and Corning filters Nos. 5543 and
3387 in the secondary, to isolate the emitted fluorescence between
450 and 470 rnp. These sensitivities and the species differences
in activities of the enzymes between monkey and rat permitted
the measurement of from 10 µg of brain, wet weight, for β-galac-
tosidase in the rat to 120 µg for β-glucuronidase in the monkey.

The quantitative histochemical studies on rabbit white matter and
the monkey cerebellar layers required a slight change in
the freeze-dried sections already in the tubes. The contents
were transferred to tubes, 10 × 75 mm, already containing 1 ml
of 2-amino-2-methyl-1-propanol-HCl and the fluorometer read-
ings were performed as already described. The reason for the
smaller incubation volume was to increase the sensitivity of the
method by reducing the total amount of glycoside in the final
fluorometer tube, thereby reducing the fluorescence of the blank.
The change was made necessary because the freeze-dried material
weighed about only 1 µg, and because the white matter, although
it weighed 1 to 2 µg, had a low activity relative to whole brain.
Comment on Analytical Procedure—The pH optimum was 3.75 in rat and pH 4.0 in monkey for β-galactosidase. These peaks were fairly sharp (Fig. 1). For β-glucuronidase the peak in both species was at 3.75, but was rather broad, and extended as low as pH 3.3 when glycine was used as the buffer (Fig. 1). The pH optimum was appreciably higher, pH 5.4, for β-glucosidase (Fig. 1). Citrate buffers were inhibitory for β-glucuronidase, but not for β-galactosidase. Acetate was not inhibitory. In kidney, with the use of the same substrate as in this study, acetate was inhibitory for β-galactosidase but not for the other two enzymes (16). The substrate concentrations for the 4-methylumbelliferone glycosides were not at their optimal levels (100 times the Kₘ) because of their insolubility, but they were well above the Kₘ and there was a negligible change of substrate concentration during incubation. The results, while probably not rigorously following zero order kinetics, were valid for the conditions noted in Table I. Whole brain homogenates were used for kinetic studies because the quantitative histochemical studies required the use of unfraccionated central nervous system tissues. The Kₘ for lactose in rat was about 100-fold that for MU-galactoside in rat and monkey (Table I). Magnesium ion was not necessary for activity, even used for kinetic studies because the quantitative histochemical /?-glucuronidase activity on dilution, presumably due to a thermostable, undialyzable inhibitor (17, 18), did not occur in the present study, perhaps because of the presence of 0.05% albumin when the homogenates were diluted beyond 1:5 and because of the presence of albumin during incubation. An alternative possibility is that the described tissue inhibitor does not occur in rat or monkey brain. Enzyme activity was linear with time for at least 4 hours for each of the three enzymes. The temperature curve showed an activity of 2.8 to 3.7% at 2°, and 24 to 38% at 38°, compared to 100% at 38°, depending on the enzyme and the species. The extremes at 25° were 24% for β-glucuronidase in the rat and 38% for β-glucuronidase in the monkey. The stability of 4-methylumbelliferone and of the three corresponding 4-methylumbelliferone glycosides was tested under a number of conditions encountered in the enzyme assays. 4-Methylumbelliferone (5 × 10⁻⁶ M) was stable for at least 3 hours at 25° in pH 10.3, 2-amino-2-methyl-1-propanol HCl in a lighted laboratory. At pH 3.75 and at pH 5.4, 4-methylumbelliferone was stable when incubated at 38° for up to 18 hours. The three glycosides were stable at pH 3.75 or pH 5.4 for 18 hours at 38° and for at least 3 hours at pH 10.3. The recovery of 4-methylumbelliferone when incubated in the presence of brain and each of the corresponding glycosides varied from 90 to 104%. Under these same conditions, except that the brain was boiled for 3 min, the glycosides did not change in fluorescence. Additionally, there was no tissue blank when active central nervous system tissue was present, so that it was not necessary to run blanks with tissue in them. The recovery of added partially purified β-galactosidase (300-fold purified) and β-glucuronidase (100-fold purified) when added to a whole brain homogenate was 103% and 94%, respectively. Because of the insolubility of central nervous system β-glucosidase, it was not possible to do a recovery experiment with β-glucosidase. The reproducibility of the methods was satisfactory. For each enzyme a dozen samples were taken from a rat brain homogenate and assayed. For β-galactosidase the mean activity was 13.7 ± 0.2 (coefficient of variation = 1.4%), for β-glucuronidase the mean was 3.10 ± 0.04 (coefficient of variation = 1.3%), and for β-glucosidase the mean was 1.42 ± 0.05 (coefficient of variation = 3.5%). Enzyme activities were expressed as millimoles per kg, wet weight, per hour.

RESULTS

Specificity of Three β-Glycosidases

These enzymes were specific with regard to the sugar moiety, although, as is well known, the aglycon portion may vary considerably in its structure (18–21). Conchie and Levvy (19) first showed the inhibition of glycosidases by the aldonolactones...
of the corresponding configuration. Complex effects of the inhibitors have, however, been reported (18, 20, 21) because of differences in species, differences in organs, and multiple forms of the same enzyme. In the present study, each aldonolactone was used in a high enough concentration to inhibit completely its corresponding glycosidase, and, under these circumstances, there was no effect (or very minimal effect) on the other two enzymes (Table II). The experiments were performed on whole brain homogenates to be certain that all active enzymes were included, because in the freeze-dried tissue (see below) whole tissue necessarily was used for quantitative histochemical distribution studies. The demonstration of total inhibition of one enzyme by its corresponding aldonolactone, coupled with no effect, or virtually none, on the other two enzymes concerned, indicated that the enzymes are different.

The $K_i$ values for β-galactosidase, β-glucuronidase, and β-glucosidase in rat were, 90, 0.85, and 19 μM, respectively; in the monkey the corresponding $K_i$ values for each enzyme were 730, 1.5, and 28. The $K_i$ values were calculated by the method of Dixon (22). The greatest potency of inhibition by the p-galactono-1,4-lactone for β-glucuronidase has been found by many investigators in other tissues. Each of the inhibitors proved to be a competitive inhibitor (Lineweaver-Burk plots).

### Solubility and Subcellular Distribution of Three Enzymes

**Solubility**—After 15 min, a water homogenate from beef brain and from monkey brain was centrifuged at 34,000 × g for 1 hour. About 50% of the β-galactosidase and β-glucuronidase activity went into the supernatant fluid, whereas only 10% of the β-glucosidase activity appeared in the supernatant fluid. Attempts to solubilize the three enzymes further by rehomogenizing the sediment with water, with 1 M NaCl, with sodium phosphate buffers (0.1 to 0.5 M, pH 6.5 to 7.5), with 0.05 to 1% bovine serum albumin, and by sonication were not successful. Making an acetone powder of the brain tissue completely solubilized the β-galactosidase and β-glucuronidase, but had no effect on the solubility of β-glucosidase (10).

Treatment of the homogenates with a non-ionic detergent, iso-octylphenoxypolyethoxyethanol (Cutsicum), increased the proportion of β-glucosidase in the supernatant fluid from 10 to 90%, while at the same time increasing the total activity by 90% and decreasing the activity in the sediment by 16%. The increased activity in the supernatant fluid was apparently in the form of micelles and not truly solubilized, since further fractionation with ammonium sulfate or acetone led to the β-glucosidase once again becoming insoluble in an aqueous medium when attempting to redissolve the precipitate in an aqueous buffer. As a consequence, the total purification achieved was only 4-fold.

**Subcellular Distribution**—The subcellular distribution of these enzymes was studied using the procedures of Brody and Bain (23), Procedure II (Table III), and of Toschi (25) (Table IV). The Toschi procedure produces a highly purified and homogeneous brain microsomal fraction, as shown by electron microscopy (26), with a minimum of tissue manipulation. The differences in methodology may account for some of the more outstanding differences between the results obtained by the two different methods. The purpose of using two independent fractionation methods was to increase the likelihood that the subcellular localization found was not an artifact, but validly represented the subcellular distribution of these enzymes. The differences were chiefly in the proportion of enzyme activity in the $S_{18,000}$ (P_{18,000}) + $S_{25,000}$ fraction. The values obtained with a motor driven (600 rpm) Teflon pestle for β-galactosidase, β-glucuronidase, and β-glucosidase were, respectively: Brody and Bain method, 25%, 19%, and 22% (Table III), Toschi method, 7%, 11%, and 12% (Table IV). That these differences were at least in part a reflection of the method of preparation was demonstrated by using a hand Teflon pestle with the Brody and Bain method, in which case the results were 5%, 6%, and 8% in the $S_{18,000}$ fraction for the enzymes, respectively. The hand pestle results resembled more nearly those obtained by the Toschi procedure. In summary, the greatest single proportion for each of the three enzymes was found in the $P_{18,000}$ fraction (Table III) or the $P_{25,000}$ fraction (Table IV). The enzymes were, therefore, chiefly mitochondrial (or lysosomal). Despite their differences in solubility the subcellular distribution of the three enzymes was very similar.
Table III

Sucrose fractionation of rat brain homogenate (83)

Rat brains were homogenized in 0.25 M sucrose at 600 rpm with a Teflon pestle and treated as described by Brody and Bain (23), their Procedure II. Assays for the enzymes are described under “Analytical Procedure.” Protein was measured by the method of Lowry et al. (24).

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total activity (\mu)mmoles/hr</th>
<th>Activity (\times 10^2)</th>
<th>Specific activity (\times 10^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\beta)-Galactosidase</td>
<td>(\beta)-Glucosidase</td>
<td>(\beta)-Glucuronidase</td>
</tr>
<tr>
<td>Homogenate</td>
<td>90</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>S(_{400})</td>
<td>89</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>P(_{400})</td>
<td>12.9</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>S(_{1000})</td>
<td>83</td>
<td>38.8</td>
<td>30.8</td>
</tr>
<tr>
<td>P(_{1000})</td>
<td>3.8</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>S(_{12,000})</td>
<td>28.6</td>
<td>13</td>
<td>15.1</td>
</tr>
<tr>
<td>P(_{12,000})</td>
<td>15.7</td>
<td>21</td>
<td>15</td>
</tr>
<tr>
<td>S(_{15,000})</td>
<td>21.8</td>
<td>21</td>
<td>15</td>
</tr>
<tr>
<td>P(_{15,000})</td>
<td>5.7</td>
<td>2.3</td>
<td>6</td>
</tr>
<tr>
<td>S(_{100,000})</td>
<td>15.2</td>
<td>6.7</td>
<td>3.8</td>
</tr>
<tr>
<td>P(_{100,000})</td>
<td>9.5</td>
<td>3.1</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Recovery (%)

| Homogenate | 94 | 84 | 76 |

\(a\) S, supernatant fluid; P, particulate fraction.

\(b\) Setting the homogenate = 100%.

\(c\) The subscripts indicate the \(g\) of centrifugation of each fraction.

\(d\) Procedure II was followed except that an 18,000 \(\times g\) centrifugation was substituted for the 23,000 \(\times g\) and, a final centrifugation at 105,000 \(\times g\) was added to enable comparison with the Toschi method.

Table IV

Sucrose fractionation of brain homogenate (83)

Rat brains were homogenized in 0.25 M sucrose and treated as described by Toschi (25). Assays for the enzymes are described under “Analytical Procedure.”

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total activity (\mu)mmoles/hr</th>
<th>Activity (\times 10^2)</th>
<th>Specific activity (\times 10^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\beta)-Galactosidase</td>
<td>(\beta)-Glucosidase</td>
<td>(\beta)-Glucuronidase</td>
</tr>
<tr>
<td>Homogenate</td>
<td>43.8</td>
<td>12.9</td>
<td>11.8</td>
</tr>
<tr>
<td>S(_{400})</td>
<td>5.8</td>
<td>1.0</td>
<td>1.2</td>
</tr>
<tr>
<td>P(_{400})</td>
<td>31.2</td>
<td>10.0</td>
<td>8.6</td>
</tr>
<tr>
<td>S(_{1000})</td>
<td>2.9</td>
<td>1.1</td>
<td>4.4</td>
</tr>
<tr>
<td>P(_{1000})</td>
<td>0.16</td>
<td>0.4</td>
<td>8.3</td>
</tr>
<tr>
<td>Recovery</td>
<td>78</td>
<td>90</td>
<td>84</td>
</tr>
</tbody>
</table>

Distribution of Three Enzymes in Central Nervous System

Before studying the more precise quantitative histochemical localization of the three enzymes, their activities in various grossly dissected parts of monkey brain were determined (Table V). In monkey brain, \(\beta\)-galactosidase was the most active, averaging 6 to 8 times more activity than \(\beta\)-glucuronidase and 2 to 4 times more than \(\beta\)-glucosidase. In white matter (internal capsule, pyramidal, inferior cerebellar peduncle), these ratios changed appreciably. \(\beta\)-Galactosidase was 4 to 5 times more active than \(\beta\)-glucuronidase, and 3 to 7 times more active than \(\beta\)-glucosidase.

Quantitative Histochemical Localization

White Fiber Tracts of Rabbit (Table VI)—The activities of the enzymes were most closely related to degree of myelination of the tract studied, even when the results were calculated on the basis of fat-free dry weight (Table VI). The largely unmyelinated fibrillar layer of the olfactory bulb showed the highest activity for each enzyme. The dorsospinocerebellar tract and the dorsal columns, which contain large heavily myelinated
fbers, generally showed the lowest activity. These results suggest that each of these enzymes is quantitatively more related to metabolism in axis cylinders than to that in myelin, or possibly in glia.

**Cerebellar Cortex and Subjacent White Matter (Table VII)—** The findings on the cerebellar layers and subjacent white matter of the monkey and rat indicated that while the inference relating degree of myelination to enzyme activity may be true for white fiber tracts, it is not invariably so when white and gray matter are compared (Table VII). In the monkey and also in the rat, β-galactosidase and β-glucuronidase showed about the same amount of activity in the subjacent white matter as in the all gray molecular layer when the results were calculated on a fat-free basis. This was not true for β-glucosidase. The molecular layer had more activity than the subjacent white matter.

More strikingly, the distribution of β-galactosidase and β-glucuronidase contrasted sharply with that of β-glucosidase. The two former enzymes were most active in the granular layer, whereas β-glucosidase was most active in the molecular layer. These contrasting findings add additional support to what is presumably a significant biological difference between β-glucosidase and the other two enzymes, at least with regard to its solubility and quantitative histochemical distribution.

**TABLE VI**

*Activity of three β-glycosidases in white fiber tracts of albino rabbit*

Assayed in sextuplicate from freeze-dried tissue as described under "Analytical Procedure." Results are expressed as millimoles of substrate hydrolyzed per kg of dry brain per hour (A) and as millimoles per kg of fat-free dry weight per hour (B).

<table>
<thead>
<tr>
<th>Tract</th>
<th>β-Galactosidase</th>
<th>β-Glucuronidase</th>
<th>β-Glucosidase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>Fibrillar layer</td>
<td>53.8</td>
<td>87.0</td>
<td>18.7</td>
</tr>
<tr>
<td>olfactory bulb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fornix</td>
<td>26.4</td>
<td>65.3</td>
<td>7.93</td>
</tr>
<tr>
<td>Pyramid</td>
<td>21.3</td>
<td>60.2</td>
<td>4.08</td>
</tr>
<tr>
<td>Optic tract</td>
<td>17.3</td>
<td>60.5</td>
<td>3.61</td>
</tr>
<tr>
<td>Dorsospinoocerebellar</td>
<td>13.7</td>
<td>66.9</td>
<td>2.52</td>
</tr>
<tr>
<td>tract</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dorsal column</td>
<td>12.1</td>
<td>50.4</td>
<td>2.87</td>
</tr>
<tr>
<td>Prior horn of</td>
<td>41.4</td>
<td>8.40</td>
<td>5.26</td>
</tr>
<tr>
<td>cervical cord</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The percentage of protein per unit of dry weight for each tract was 61.8, 40.4, 30.8, 28.6, 20.4, and 23.9%, respectively.*

**TABLE VII**

*Activity of three β-glycosidases in layers of monkey and rat cerebellar cortex and subjacent white matter*

Assayed as described in Tables I and IV. Results are expressed as millimoles of substrate hydrolyzed per kg of dry brain per hour. Two or three animals of each species were used for each mean value.

<table>
<thead>
<tr>
<th>Cerebellar layer</th>
<th>β-Galactosidase</th>
<th>β-Glucuronidase</th>
<th>β-Glucosidase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monkey</td>
<td>Rat</td>
<td>Monkey</td>
</tr>
<tr>
<td>Molecular</td>
<td>8.33 (11.6)</td>
<td>39.3 (54.9)</td>
<td>2.16 (3.02)</td>
</tr>
<tr>
<td>Granular</td>
<td>24.7 (51.9)</td>
<td>117.2 (151.5)</td>
<td>5.86 (7.56)</td>
</tr>
<tr>
<td>White matter</td>
<td>5.53 (12.4)</td>
<td>24.9 (56.0)</td>
<td>2.15 (4.83)</td>
</tr>
</tbody>
</table>

*The figures in parentheses are the activities expressed as millimoles of substrate hydrolyzed per kg of fat-free dry weight per hour.*

DISCUSSION

A modification of an existing method (11) is described in which glycosidases are measured when the aglycon, 4-methylumbellifereone, is released from the corresponding 4-methylumbellifereone glycosides to permit the measurement of β-galactosidase, β-glucuronidase, and β-glucosidase in as little as 1 μg of neural tissue. Criticisms of methods utilizing 4-methylumbellifereone as the aglycon have been described (18). These criticisms were that the method is too exacting because of the instability of 4-methylumbellifereone solutions on exposure to light, particularly at the alkaline pH required for fluorescence, and because of the high reading given by the substrate MU-glucuronide at concentrations necessary to saturate the enzyme. In the example given the final concentration of MU-glucuronide during incubation was 0.02 mM (18), yet in the present paper a 1 mM substrate concentration was used during incubation without producing excessively high blank readings. The instability to light when using 2-amino-2-methyl-1-propanol-HCl as the diluent for reading at an alkaline pH presents no problem. As late as 1964, similar criticisms, without additional data, of the 4-methylumbellifereone method were still being published (28). For this reason, it was believed necessary to present additional data concerning stability and to discuss these data. The 4-methyl-umbellifereone method is not only simple and sensitive but generally useful for routine assays.

With the corresponding aminolactones as inhibitors, it was shown with a high degree of probability that each of these enzymes is separate and distinct from the other two. This finding was reinforced by showing that β-glucosidase is insoluble in an aqueous medium, whereas the other two enzymes are 50% soluble; that the pH optimum for β-glucosidase is different from that of the other two enzymes; and that the quantitative histochemical distribution of β-glucosidase differs strikingly in layers of the cerebellum when compared with that of the other two enzymes. Paper II (29) of this series will discuss in detail the localization of these enzymes in individual large nerve cell bodies and the possible relevance of this finding to their lysosomal localization.

The separation of subcellular particles from brain is much less satisfactory than that for other organs, e.g. liver. Despite the uncertainty introduced by this difficulty in obtaining cleanly separated particles, it is clear that the three β-glycosidases being considered have an almost identical subcellular distribution, even though β-glucosidase is much more insoluble (membrane bound? as it appears to be in liver (30)) than is β-galactosidase or β-glucuronidase. β-Galactosidase and β-glu-
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S-glycosidases considered in this paper is lysosomal in origin. It is probable that β-glucosidase is also a lysosomal enzyme, but in brain it is more tightly bound than the other two glycosidases. β-Glucosidase does manifest structure-linked latency (2) characteristic of lysosomal enzymes. It is, therefore, difficult to be certain, but it appears that a part of or all of each of the three β-glycosidases considered in this paper is lysosomal in origin.

Work on central nervous system enzymes associated with glycolipid metabolism is recent and developing rapidly. Gatt (31), Gatt and Rapport (4), and Frohwein and Gatt (32) have demonstrated, with relatively impure enzyme preparations, brain glycosidases that have the apparent capability of degrading monosialoganglioside to ceramide and glucose. Work from this laboratory reporting on the use of glycolipids as substrates will be reported in Paper III (10). Hajra et al. (7) have isolated brain glycosidases that have the apparent capability of degrading galactosylgalactosylglucosylceramide occurs in brain. These were not central nervous system enzymes (33, 34), although glycolipids have not been explored yet.

The recent and rapid growth of knowledge of the action of glycosidase signifies that more work from more laboratories will be required to firmly demonstrate and clearly elucidate the enzymatic mechanisms involved and the biological significance of these enzymes. This is especially true when neural tissue itself is being studied. The present paper and the next two of this series (10, 29) will, we hope, contribute to the further understanding of these problems in the neural tissue.

The recent findings of glycerylgalactosides in brain (35–38), monoalkylmonoacylglyceryl galactoside and diacyl-glyceryl galactoside, suggest that a galactosidase may be involved in their metabolism, but the metabolic pathways of glycolipidolipids have not been explored yet.

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