

Hexosaminidase C in Brain Affected by Tay-Sachs Disease

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MINAMI, R., NAKAMURA, F., OYANAGI, K. and NAKAO, T. *Hexosaminidase C in Brain Affected by Tay-Sachs Disease.* Tohoku J. exp. Med., 1981, 133 (2), 175-185 — Hexosaminidase (Hex) C could be detected in human brains by DEAE cellulose column chromatography, when N-acetyl- β -D-glucosaminidase activity was determined at pH 5.5. In the chromatographic pattern, the peak of Hex C was always higher than that of Hex A in control prenatal brains and conversely lower in postnatal brains. Hex C was not detected in the brains obtained from two cases of Tay-Sachs disease. In addition, the content of Hex C in the brains of two other Tay-Sachs cases was lower than that of the control. The observation suggests that the absence or reduction of Hex C may have some relation with biochemical defects in Tay-Sachs disease, although Hex C does not share common subunit structures with Hex A and the role of Hex C with respect to GM₂-ganglioside degradation remains unknown yet. — hexosaminidase C; Tay-Sachs disease; DEAE cellulose column chromatography

Hexosaminidase (Hex) in human brain consists of two major components, A and B. In addition, two minor components, C and S, have been described in brains obtained from normal, Tay-Sachs and Sandhoff diseases (Braidman et al. 1974a; Besley and Broadhead 1976; Beutler and Kuhl 1977; Potier et al. 1979). Hex C differs from Hex A, B and S in various properties; extralysosomal localization, large size of the molecule, neutral pH optimum, different substrate specificity and absence of immunological relationship (Penton et al. 1975). Therefore, the existence of Hex C in tissues affected by Tay-Sachs disease has been the subject of various discussions (Hooghwinkel et al. 1972; Poenaru and Dreyfus 1973; Bradiman et al. 1974b; Penton et al. 1975; Van-Cong et al. 1975; Reuser and Galjaad 1976; Swallow et al. 1976).

The present paper concerns with Hex C activities in brains obtained from four cases of Tay-Sachs disease. In addition, difference in Hex C activity between control prenatal and postnatal brains will be reported.

MATERIALS AND METHODS

Tissue sample

Pathological brain tissues were obtained from a therapeutically aborted fetus (23 weeks of pregnancy) and three patients (a 33-month-old girl, a 34-month-old girl and a 42-

month-old boy) with Tay-Sachs disease. Control fetal brains between 20 and 24 weeks of gestation were obtained from therapeutic abortions for psychosocial reasons. Control brains between 2 months and 77 years of age were obtained post mortem from patients with no apparent central nervous system disease. All these tissue specimens were immediately frozen after autopsy and stored at -20°C for two months to five years prior to analysis.

Enzyme preparation

Frozen pieces of brain were homogenized in 5–20 volumes of ice-cold deionized water. The homogenate was sonicated for 1 min (Branson, Sonifier, Cell Disruptor 200), then centrifuged at $100,000\times g$ for 60 min. The supernatant was used as enzyme source.

Enzyme assay

Hex activity was determined using 4-methylumbelliferyl (4MU)-2-acetamido-2-deoxy- β -D-glucopyranoside (4MU-glucosaminide) and -galactopyranoside (4MU-galactosaminide) (Hooghwinkel et al. 1972) (Koch-Light Laboratories, Ltd.). PH optima were examined with the reaction mixtures consisting of 0.1 ml of 0.1 M citrate-phosphate buffer of varied pH, 0.1 ml of enzyme sample containing 0.1% human serum albumin (Hex activity-free) and 0.4 ml of 1 mM 4MU-glucosaminide or 0.5 mM 4MU-galactosaminide. The reaction was stopped by the addition of 3 ml of 0.25 M glycine buffer, pH 10.7. Fluorescence was read immediately with a Hitachi spectrophotofluorometer (Model MPF-4) with an excitation wavelength 365 nm, emission wavelength 450 nm. Protein was assayed according to the method of Lowry et al. (1951).

Ion exchange chromatography

Whatman DEAE cellulose (Type DE-52) was equilibrated in 10 mM citrate-phosphate buffer, pH 6.0 and packed in a column (1.0×10.0 cm). After the sample (1.0 ml of enzyme preparation) was applied to the column, the column was washed with 60 ml of the equilibration buffer and then eluted with a linear KCl gradient (0–0.2 M) in 120 ml of the same buffer. Fractions of 3.0 ml were collected at a flow rate of 50 ml/hr. Chromatography was carried out at 4°C . Each fraction was assayed for Hex activity as follows: 0.1 ml of the fraction was incubated with 0.5 ml of 0.8 mM 4MU-glucosaminide in 0.1 M citrate-phosphate buffer at pH 4.5 and 5.5 for 60 min.

Concanavalin (Con) A-Sepharose affinity chromatography

Samples of control and Tay-Sachs brains (0.5 ml of enzyme preparation) were applied to a column (1×5 cm) of Con A-Sepharose according to the method of Swallow et al. (1976). After sample application, the column was washed with 15 ml of 0.1 M acetate buffer, pH 6.0 containing 1 M NaCl, 0.01 M MnCl_2 , 0.01 M CaCl_2 , 0.01 M MgCl_2 and then eluted with 30 ml of 0.5 M methyl- α -D-mannoside dissolved in the above buffer. Fractions of 3.0 ml were collected at a flow rate of 14 ml/hr. Chromatography was carried out at room temperature. Each fraction was assayed for Hex activity as mentioned above.

Electrophoresis

Horizontal starch-gel electrophoresis was carried out as described by Swallow et al. (1976). The gel was prepared from one thirtieth dilution of bridge buffer (0.22 M tris and 0.086 M citric acid) containing 11% starch and adjusted to pH 5.7. Samples were inserted into the gel using a small piece of filter paper and electrophoresis was performed at 30 V/cm for 4 hr at 4°C .

Staining

Hex was located using 4MU-glucosaminide as substrate which was dissolved in 0.1 M citrate-phosphate buffer, pH 5.5. The product was visualized under ultraviolet light (Swallow et al. 1976).

RESULTS

Ion exchange chromatography

DEAE cellulose column chromatography of the samples from control brains showed two major isoenzymes (Hex B and A), when N-acetyl- β -D-glucosaminidase

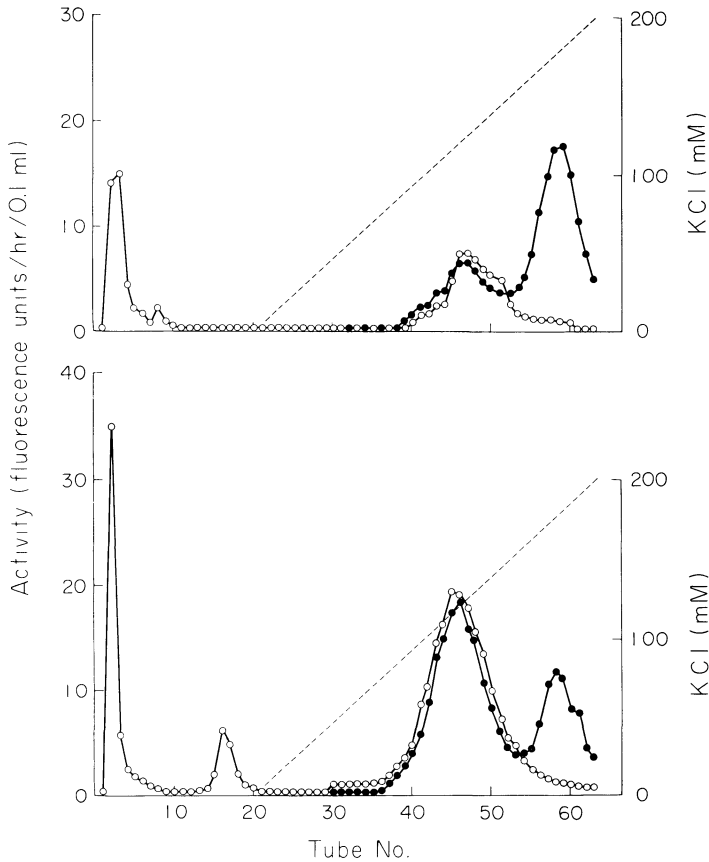


Fig. 1. Fractionation of hexosaminidase isoenzymes in the control brains by DEAE cellulose column chromatography. Upper: 20 weeks of pregnancy. Lower: 2 months old. The plotted relative activities were the fluorescence values. N-Acetyl- β -D-glucosaminidase activity was measured at pH 4.5 (○) and pH 5.5 (●).

was assayed at pH 4.5. On the other hand, another peak was found in a fraction which was eluted at higher salt concentration than that required for Hex A, when the assay was carried out at pH 5.5 (Figs. 1, 2). The third peak was always higher than that of Hex A in the prenatal brains derived from various gestation ages examined and, on the contrary, always lower in the postnatal brains. The elution profiles for the Tay-Sachs brains were shown in Figs. 3 and 4. The third peak was absent in the elution profiles for the brains obtained from fetal Tay-Sachs disease and a case of Tay-Sachs disease. The amounts of Hex A, B and the third peak component in the control and Tay-Sachs brains were calculated on the basis of total fluorescence activity of respective Hex peak areas of the eluate fractions. In the two Tay-Sachs brains, in which the third peak was present, the ratio of the amount of the third peak component to those of the other peak components was found to be lower than in the control brains.

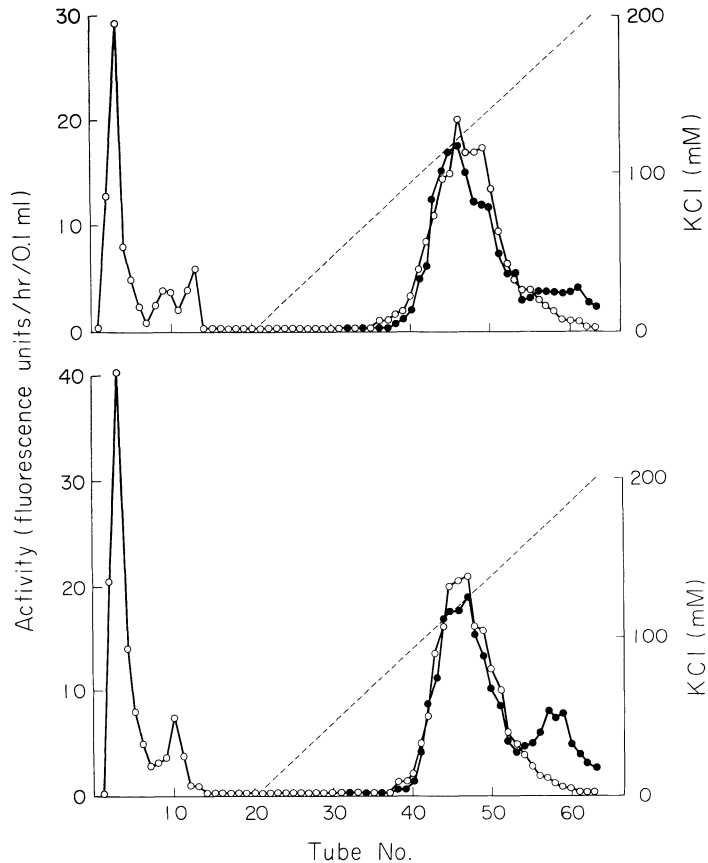


Fig. 2. Fractionation of hexosaminidase isoenzymes in the control brains by DEAE cellulose column chromatography. Upper: 8 years old. Lower: 77 years old. The plotted relative activities were the fluorescence values. N-Acetyl- β -D-glucosaminidase activity was measured at pH 4.5 (○) and pH 5.5 (●).

Con A-Sepharose affinity chromatography

Column chromatography on Con A-Sepharose revealed that more than 50% of total N-acetyl- β -D-glucosaminidase activity in the control fetal brain as assayed at pH 5.5 was unbound to Con A-Sepharose, while almost all of the activity in fetal Tay-Sachs brain was bound to Con A-Sepharose (Fig. 5). Swallow et al. (1976) had confirmed the binding of Hex A and B to Con A-Sepharose, and unbinding of Hex C. The Hex C from fetal brain which remained unbound to Con A-Sepharose was again chromatographed on DEAE cellulose column. As shown in Fig. 6 a small peak of N-acetyl- β -D-glucosaminidase as assayed at pH 4.5, was observed at the same salt concentration as required to elute a large peak as assayed at pH 5.5. Therefore, the peak detected by assay of N-acetyl- β -D-glucosaminidase at pH 5.5 on DEAE cellulose column chromatography was identified as Hex C.

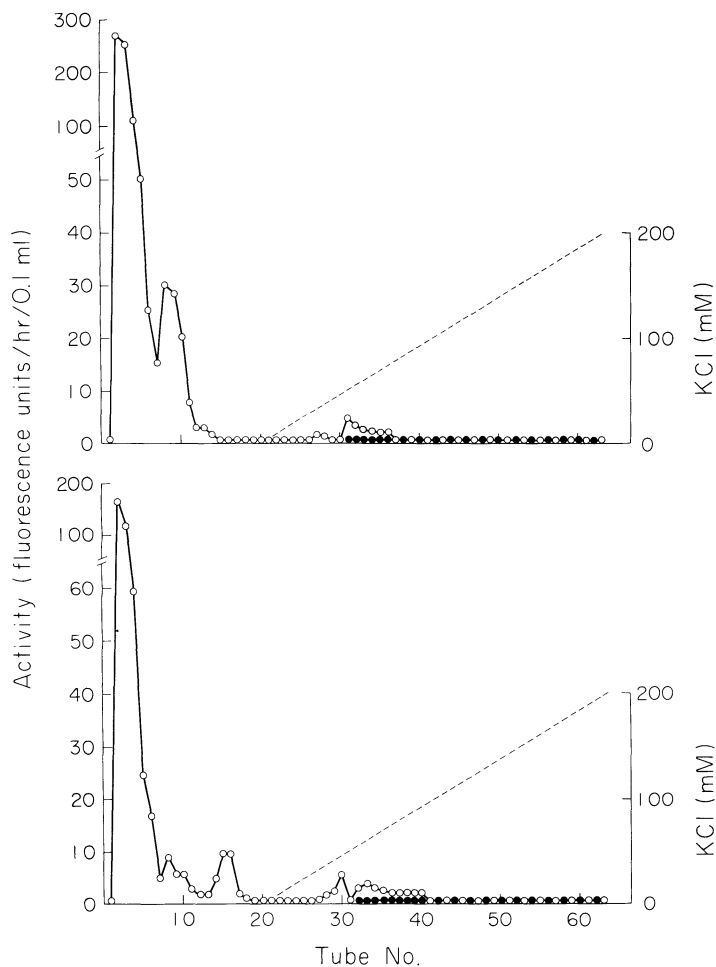


Fig. 3. Fractionation of hexosaminidase isoenzymes in the brains obtained from Tay-Sachs disease by DEAE cellulose column chromatography. Upper: 23 weeks of pregnancy. Lower: 33 months old. The plotted relative activities were the fluorescence values. N-Acetyl- β -D-glucosaminidase activity was measured at pH 4.5 (○) and pH 5.5 (●).

Electrophoresis

When stained at pH 5.5. with 4MU-glucosaminide, a large amount of Hex C was found in fetal brain and a lesser amount of Hex C in child brain (Fig. 7). Prenatal brain had a higher level of Hex C than postnatal brain when examined by electrophoresis. This observation is consistent with the data of DEAE cellulose column chromatography.

Properties of Hex A, B and C in the fetal brain

Individual peak fractions on DEAE and Con A-Sepharose chromatographies of the fetal brain sample were pooled separately and dialyzed against deionized

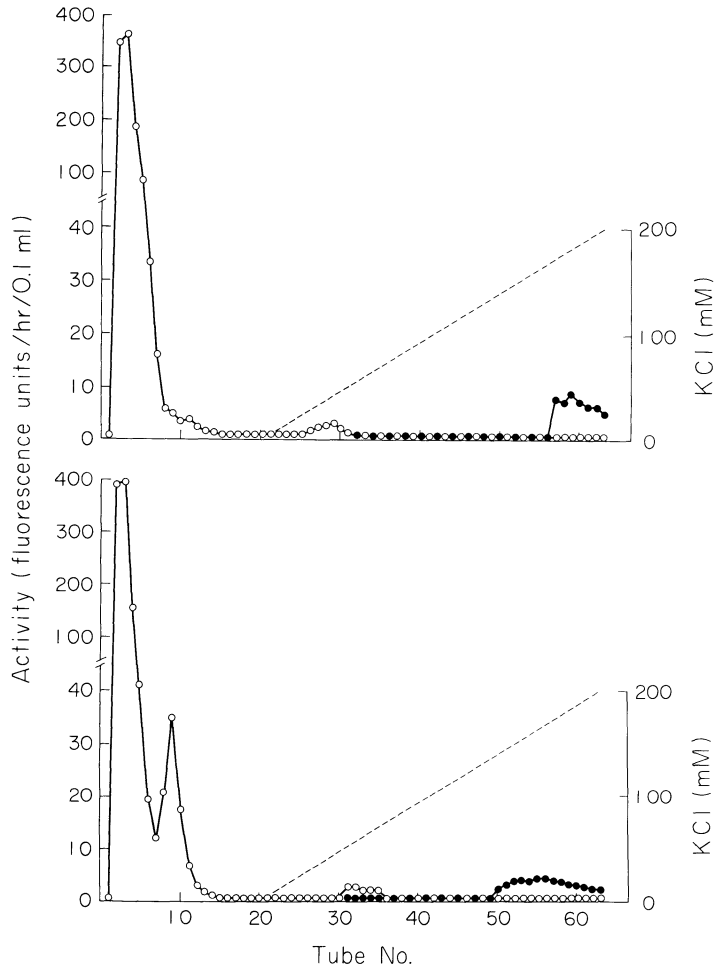


Fig. 4. Fractionation of hexosaminidase isoenzymes in the brains obtained from Tay-Sachs disease by DEAE cellulose column chromatography. Upper: 34 months old. Lower: 42 months old. The plotted relative activities were the fluorescence values. N-Acetyl- β -D-glucosaminidase activity was measured at pH 4.5 (o) and pH 5.5 (●).

water overnight at 4°C, then lyophilized. After lyophilization, each component was dissolved in a small volume of ice-cold deionized water. An optimal pH for Hex C from both chromatographies showed 5.5 with 4MU-glucosaminide (Fig. 8); this disagrees with the previous report (Penton et al. 1975). Hex C showed a markedly lower activity toward 4MU-galactosaminide as compared with Hex A and B.

Total Hex activities of the brains obtained from controls and Tay-Sachs diseases

N-Acetyl- β -D-glucosaminidase activity was assayed with 10–20 μ g protein of the enzyme sample and N-acetyl- β -D-galactosaminidase, with 20–40 μ g protein. Total N-acetyl- β -D-glucosaminidase activities of the postnatal brains at both pH

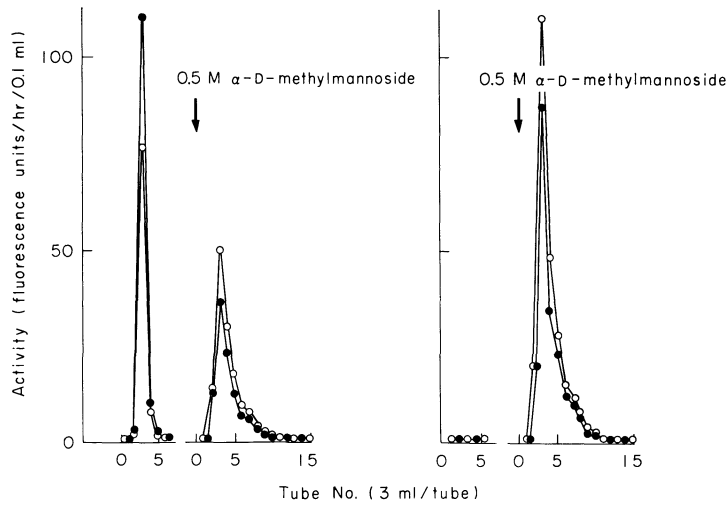


Fig. 5. Con A-Sepharose affinity chromatography of brain hexosaminidase from the control fetus and the fetus with Tay-Sachs disease. Left: Control, 24 weeks of pregnancy. Right: Tay-Sachs disease, 23 weeks of pregnancy. The plotted relative activities were the fluorescence values. N-Acetyl- β -D-glucosaminidase activity was measured at pH 4.5 (\circ) and pH 5.5 (\bullet).

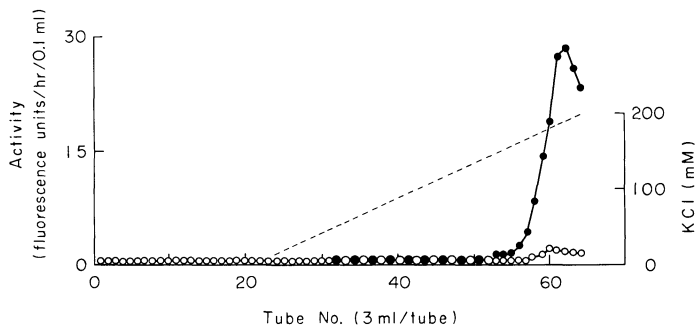


Fig. 6. DEAE cellulose column chromatography of hexosaminidase (Hex C) unbound to Con A-Sepharose prepared from control fetal brain. The plotted relative activities were the fluorescence values. N-Acetyl- β -D-glucosaminidase activity was measured at pH 4.5 (\circ) and pH 5.5 (\bullet).

4.5 and 5.5 were larger than those of the prenatal brains and the ratios of the activities at pH 5.5 and 4.5 in the postnatal brains were smaller than those in the prenatal brains (Fig. 9). Tay-Sachs displayed the increased activities. In contrast to the cases of the control fetal brain, however, N-acetyl- β -D-glucosaminidase activity at pH 5.5. was smaller than that at pH 4.5 in the fetal Tay-Sachs brain.

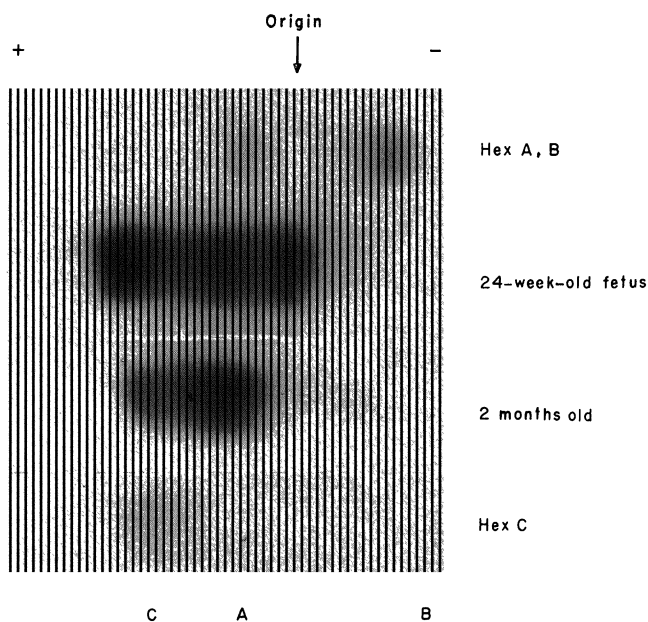


Fig. 7. Starch gel electrophoresis of N-acetyl- β -D-glucosaminidase at pH 5.5 in the brain extracts.

Hex A, B and C were prepared from DEAE cellulose column chromatography of the fetal brain.

DISCUSSION

Hex C was first observed in human brain tissues by Hooghwinkel et al. (1972). Thereafter, Hex C has been separated from Hex A and B using electrophoresis. A few papers (Braidman et al. 1974a; Overdijk et al. 1975) reported that Hex C could not be separated by ion exchange chromatography on DEAE cellulose, because Hex C appeared to undergo some change under this condition. In the present study, Hex C could be separated from Hex A and B by DEAE cellulose column chromatography.

The peak of Hex C in the elution profile was higher than that of the Hex A in the prenatal brains and lower in the postnatal brains. Poenaru and Dreyfus (1973) also showed by electrophoresis that Hex C in extracts from embryonic tissues was more abundant than that from adult tissues. On the other hand, Hex S, which is one of the minor components of Hex isoenzymes, could not be detected in the control or Tay-Sachs brains as reported by Beutler and Kuhl (1977).

The existence of Hex C in tissues affected by Tay-Sachs disease has only been discussed (Hooghwinkel et al. 1972; Poenaru and Drefyus 1973; Braidman et al. 1974b; Penton et al. 1975; Van-Cong et al. 1975; Reuser and Galjaad 1976; Swallow et al. 1976), because it is difficult to separate Hex C from Hex S by electrophoresis and Hex C activity appears to be highly labile while Hex A and B are quite stable (Poenaru and Dreyfus 1973; Penton et al. 1975). We have experienced, however,

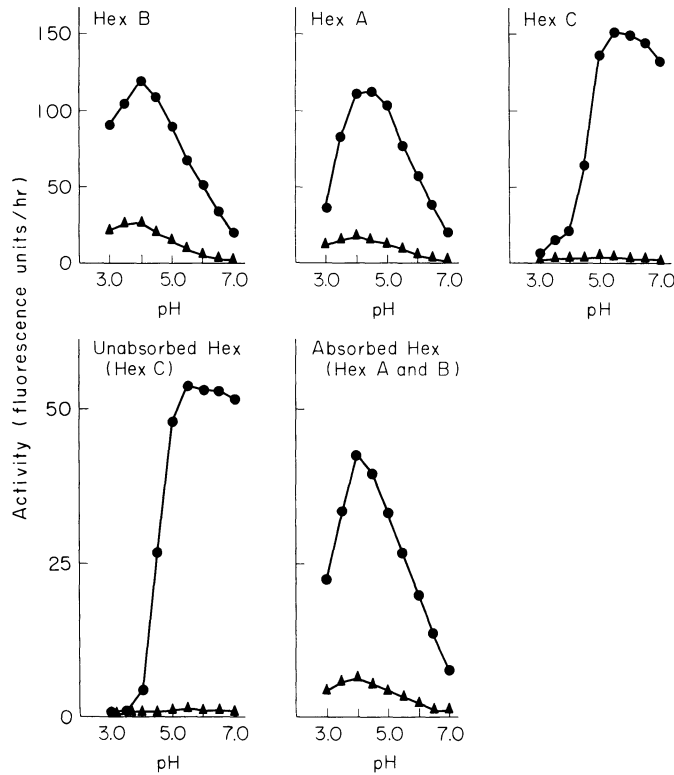


Fig. 8. pH activity profiles of hexosaminidase components prepared from the control fetal brain.

Upper: pH activity profiles of hexosaminidase A, B and C prepared from DEAE cellulose column chromatography.

Lower: pH activity profiles of Hex absorbed and unabsorbed in Con A-Sepharose.

●—●, N-acetyl- β -D-glucosaminidase; ▲—▲, N-acetyl- β -D-galactosaminidase.

that Hex C was stable for 6 months when stored at -70°C . Hex C was not detected in the brains obtained from two cases of Tay-Sachs disease. Also, the amount of Hex C in the brains of two other Tay-Sachs cases were lower than that of the control. Hex C was absent in the fetal Tay-Sachs brain, whereas a considerable amount of Hex C was present in all the control fetal brains examined. It is of interest to note that clinically the Tay-Sachs patient without Hex C activity had more trouble in seizures as compared with the Tay-Sachs patients with Hex C activity, although there was no difference between GM_2 -ganglioside accumulation in their brains (unpublished data).

Hex C seems to play a more important role in prenatal brain than in postnatal brain. Also no or diminished Hex C activity may represent a characteristic of the Tay-Sachs disease, although the role of Hex C in relation to GM_2 -ganglioside degradation remains as yet unknown and Hex C is different genetically and structurally from Hex A.

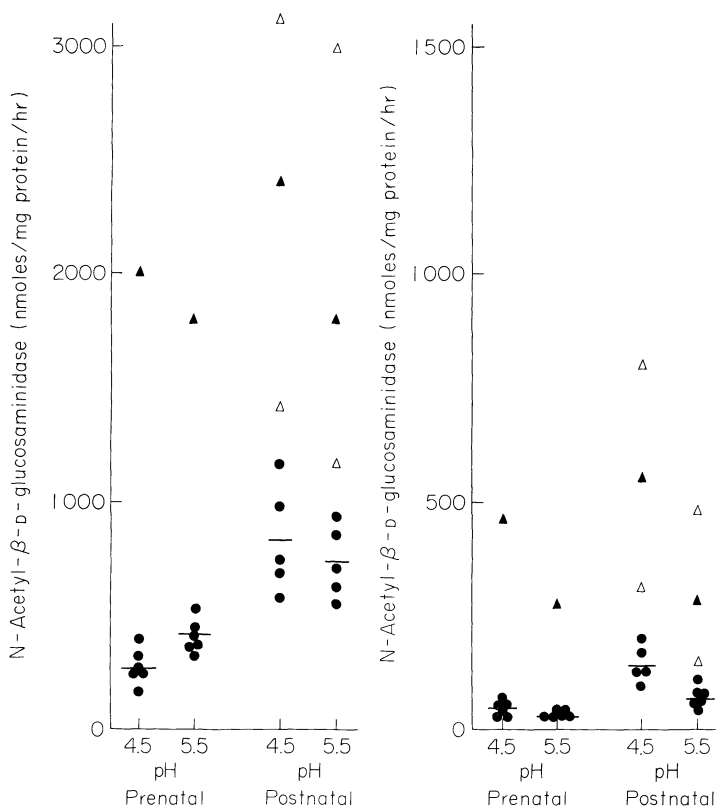


Fig. 9. Activities of hexosaminidase in the brains obtained from control individuals and patients with Tay-Sachs disease (2 months to 8 years of age). Activities were measured with 0.1 M citrate-phosphate buffer at pH 4.5 and 5.5. ●, control individuals; △, Tay-Sachs patients with Hex C activity; ▲, Tay-Sachs patients without Hex C activity.

Acknowledgments

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