the pressure range 80 to 180 kbar at 1000°C by Ringwood and Major (*19*) and at 800°C by Akimoto and Syono (*20*). Ito *et al.* (*21*) also reported that $MgSiO_3$ (clinoenstatite) decomposes to $Mg_2SiO_4$ ($\beta$-phase) plus $SiO_2$ (stishovite) at a pressure between 200 and 280 kbar at 1000°C and that $\beta$-$Mg_2SiO_4$ transforms into $\gamma$-$Mg_2SiO_4$ (spinel) at higher pressure. This last pressure reported by Ito *et al.* (*21*) seems to be overestimated. A pressure of 150 kbar is much more consistent with the other studies made on the phases in the $MgO$-$SiO_2$ system (*22*). Although recent studies (*19–21*) do not support the claim by Sclar *et al.* (*23*) that $MgSiO_3$ (clinoenstatite) decomposes to forsterite plus stishovite, they do confirm their conclusion that $MgSiO_3$ decomposes to $Mg_2SiO_4$ plus $SiO_2$. Sclar *et al.* (*23*) estimated the pressure for this transformation to be 125 kbar. With subsequent revision of the fixed point pressure scale, this value should perhaps be lowered 15 percent. This is generally consistent with our conclusion that Ito *et al.* (*21*) have overestimated the pressure. In any case, all of these results indicate that $(Mg,Fe)SiO_3$ decomposes to $(Mg,Fe)_2SiO_4$ plus $SiO_2$ under pressure at 1000°C.

On the basis of the experimental work on the olivines and pyroxenes described above and of work described elsewhere (*24*), pressure-composition phase diagrams for the $FeO$-$SiO_2$ and $MgO$-$SiO_2$ systems are proposed in Figs. 2 and 3, showing that separate oxides of $(Fe,Mg)O$ and $SiO_2$ (stishovite) are the stable phases in the system of $FeO$-$MgO$-$SiO_2$ under high pressure (>250 kbar) and high temperature (>1000°C). We believe that the mineralogy of the earth's mantle below 650 km consists mainly of magnesiowustite [$(Mg,Fe)O$] and stishovite.

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## Deficient Ganglioside Biosynthesis: A Novel Human Sphingolipidosis

Abstract. *An unusual lipid storage disease is characterized by the accumulation of hematoside ($G_{M3}$) in the patient's liver and brain. In contrast to the other sphingolipidoses, the accumulation of $G_{M3}$ is not the result of a defective catabolic reaction, but is the first disorder caused by a deficiency in ganglioside biosynthesis to be described in man.*

A group of clinically distinct disorders characterized by the accumulation of glycosphingolipids in various tissues have been described chemically and enzymatically (*1*). Several of these disorders (*2*) involve the accumulation of gangliosides $G_{M1}$ and $G_{M2}$ (*3*), and such storage has been shown to be due to defects in the catabolism of these compounds (*4*). Recently, it has been possible to document for the first time the accumulation of $G_{M3}$ and the virtual absence of higher ganglioside homologs in the brain and liver of a patient with a presumed sphingolipidosis (*5*). We report here that the patient's $G_{M3}$ (hematoside) accumulation was not due to the defective catabolic reaction

$$G_{M3} + H_2O \rightarrow CDH + NAN$$

but was the result of a deficient ganglioside biosynthetic enzyme, UDP-GalNAc : $G_{M3}$ *N*-acetylgalactosaminyltransferase (*6*) which catalyzes the following reaction:

$$G_{M3} + \text{UDP-GalNAc} \rightarrow G_{M2} + \text{UDP}$$

This is the first description of a defect in ganglioside biosynthesis in humans.

The clinical and pathological aspects of this patient's disease have been described (*7*). When first seen at the University of Maryland Hospital, this 1-month-old male had poor physical and motor development and frequent seizures. The first child of young, unrelated, Jewish parents (mother 22 and father 25 years of age), he weighed 3.5 kg at birth after a 36-week gestation. He was limp and unresponsive, with coarse facies, macroglossia, gingival hypertrophy, squat hands and feet, flexor contractures of the fingers, thickened, loose hirsute skin, large inguinal hernia, hepatosplenomegaly, and normal fundi. Death at 3½ months followed a series of bronchopneumonic episodes. A maternal uncle had a similar disorder and died at 2½ months of age. These features suggested $G_{M1}$ gangliosidosis, which was ruled out by the finding of normal $\beta$-galactosidase activity in leukocytes and in a liver biopsy. The activities of acid phosphatase, $\beta$-glucosidase, $\beta$-*N*-acetylhexosaminidase, $\alpha$-fucosidase, $\alpha$-mannosidase, and arylsulfatase A were normal. The diagnosis of $G_{M3}$ gangliosidosis was established by an accumulation of $G_{M3}$ (and $G_{D3}$) in postmortem samples of brain and liver, which was demonstrated by thin-layer chromatographic analysis. The content of $G_{M3}$ and $G_{D3}$ in the patient's brain tissue was 1.6 and 0.8 nmole per milligram of protein compared to values of 0.46 and 0.34 nmole per milligram of

protein in control brain tissue (*7*). Higher ganglioside homologs were absent from brain and decreased in liver (*5, 7*). Overall, there was an 80 percent reduction of total ganglioside sialic acid in the brain (*7*).

$G_{M3}$ sialidase was assayed in postmortem samples of frozen brain with the use of [$^{14}$C]NAN $G_{M3}$ which had been prepared biosynthetically from CDH and CMP-[$^{14}$C]NAN (New England Nuclear) (*8*). The radioactive product, purified by thin-layer chromatography, was diluted to a final specific activity of 1900 count/min per nanomole of NAN with nonradioactive $G_{M3}$. Using a previously described method (*9*), we were able to show significant $G_{M3}$ sialidase activity in homogenates of this frozen brain under optimum conditions for $G_{M3}$ hydrolysis. The enzyme activity present was similar to that in samples of normal brain that had been frozen for a similar period (Table 1). In addition, a deficiency in $G_{M3}$-sialidase activity would not explain the virtual absence of higher gangliosides in the patient's brain (*7*).

Since the absence of higher gangliosides and the accumulation of $G_{M3}$ could not be attributed to a deficient catabolic enzyme, the activities of four glycosyltransferases involved in ganglioside biosynthesis were measured (*10*). There were significant activities (picomoles per milligram of protein per hour) of UDP-Gal : $G_{M2}$ galactosyltransferase ($109 \pm 16$), of CMP-NAN : CDH sialytransferase ($32 \pm 6$), and of CMP-NAN : $G_{M1}$ sialyltransferase ($47 \pm 8$) in homogenates of the patient's brain. These activities varied in control brain homogenates from 22 to 32, 22 to 56, and 20 to 25 pmole

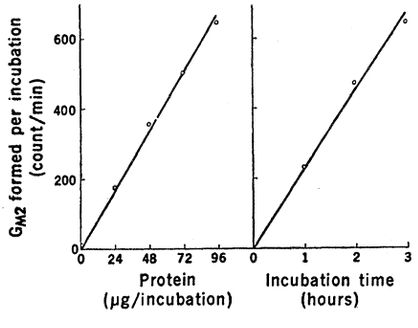


Fig. 1. Effect of time and protein content on the assay of UDP-GalNAc : $G_{M3}$ *N*-acetylgalactosaminyltransferase activity from human brain. Enzyme activity was assayed as described in Table 2, except that brain protein (left panel) or incubation time (right panel) varied.

Table 1. $G_{M3}$ sialidase activity in brain of normals and patient with $G_{M3}$ gangliosidosis. Cortical gray matter was homogenized in 5 volumes (weight to volume) of 0.25*M* sucrose containing 0.1 percent mercaptoethanol. Incubations contained 100 to 250 μg of protein, 125 m*M* citrate-phosphate buffer (*p*H 3.8), and 10 nmole of [$^{14}$C]NAN $G_{M3}$ (19,000 count/min) in a total volume of 200 μl. Incubations were carried out for 2½ hours at 37°C and assayed (*9*); activities of enzyme preparations that were boiled and activities at zero time were measured and used to correct the assays.

| Subject | Age | [$^{14}$C]NAN (pmole mg$^{-1}$ protein hr$^{-1}$) |
|---|---|---|
| Controls | 6 months | 124 |
| | 18 months | 149 |
| | Adult | 150 |
| Patient | 3½ months | 115 |

mg$^{-1}$ hr$^{-1}$, respectively. These three glycosyltransferase activities were also detected in a homogenate of the patient's liver. However, no *N*-acetylgalactosaminyltransferase activity could be demonstrated in the liver homogenate, and this glycosyltransferase activity (Table 2) was significantly lower in the patient's brain homogenate compared to those of normal brains (11 percent) or of brains from patients with known sphingolipidoses (25 percent). When homogenates of normal brains and the patient's brain were assayed for *N*-acetylgalactosaminyltransferase activity separately and compared to an admixture, theoretical amounts of $G_{M2}$ product were obtained as follows (count/min), 396 (normal) and 59 (patient) separately, and 404 (admixture). Thus, the abnormality appears to be due to a deficiency in *N*-acetylgalactosaminyltransferase activity and not to the presence of an enzyme inhibitor or absence of an enzyme activator. The enzyme assay with normal brain homogenate was linear with protein content and time of incubation (Fig. 1).

Deficiency of UDP-GalNAc : $G_{M3}$ *N*-acetylgalactosaminyltransferase activity in the brain and liver of the patient is consistent with the decrease in higher gangliosides observed in these tissues (*5, 7*), because this glycosyltransferase catalyzes a key step in the elongation of the oligosaccharide chain of these glycolipids. In contrast, a deficient catabolic enzyme would not explain such a reduction. The variations in other glycosyltransferase activities may be related to developmental changes in ganglioside biosynthesis that have been observed (*11*). We have observed that glycosyltransferase activities are elevated in human fetal brain (*12*). A second possibility is that there may be some compensating regulatory mechanism whereby abnormal accumulation or absence of gangliosides would lead to decreases or increases in their biosynthetic enzymes. The underlying cause of the UDP-GalNAc : $G_{M3}$ *N*-acetylgalactosaminyltransferase deficiency in this patient remains obscure. That a maternal uncle died of what appears to be a similar disorder points to the likely genetic nature of this disease; nevertheless other mechanisms involving viruses might be considered. A decrease in *N*-acetylgalactosaminyltransferase activity has been observed in virus-transformed mouse (*13*) and hamster (*14*) cells. Viral transformation of human cells in culture resulted in similar deficiencies in ganglioside biosynthetic enzymes (*12*).

Information concerning the functioning of gangliosides is quite limited. However, the far-ranging and devastat-

Table 2. UDP-GalNAc : $G_{M3}$ *N*-acetylgalactosaminyltransferase activities in brain homogenates from controls and a patient with $G_{M3}$ gangliosidosis. Tissue samples were homogenized in 9 volumes of 0.25*M* sucrose containing 0.1 percent β-mercaptoethanol. The reaction mixtures and incubation conditions were as follows: $G_{M3}$, 50 nmole; UDP-[$^{14}$C]-GalNAc (20,000 count/min per nanomole), 10 nmole; sodium cacodylate, *p*H 7.0, 2.5 μmole; $MnCl_2$, 1 μmole; Triton X-100, 200 μg; homogenate, 10 μl in a final volume of 50 μl. Similar reaction mixtures were prepared without added glycolipid acceptors. The reaction mixtures were incubated for 3 hours at 37°C, and the reaction products were isolated by Sephadex column chromatography (*10*). The $G_{M2}$ reaction product of the *N*-acetylgalactosaminyltransferase assays was further purified by thin-layer chromatography on silica gel G in the solvent system consisting of chloroform, methanol, 10 percent $NH_4OH$, and 4 percent $CaCl_2$ (120 : 70 : 16 : 1 by volume). The incorporation of radioactive sugars into glycolipids was then determined by liquid scintillation counting and corrected for incorporation due to controls (enzyme preparations that had been boiled). Preparation of the glycolipid acceptor and sources of the other reagents have been described (*10*).

| Brain sample | Age of subject | Total $G_{M2}$ synthesis* (pmole mg$^{-1}$ protein hr$^{-1}$) |
|---|---|---|
| Normal† | 9 months | $124 \pm 11$ |
| Tay-Sachs† | 1.5 years | $55 \pm 3.9$ |
| Tay-Sachs† | 1.5 years | $55 \pm 3.9$ |
| Krabbe† | 1 year | $54 \pm 3.0$ |
| Patient‡ | 3½ months | $14 \pm 6.6$ |

* Activities are the mean ± the standard deviation of three determinations. Controls with enzyme preparation that had been boiled varied from 3.5 to 8.0 pmole per milligram of protein per hour. Endogenous activities varied from 2.7 to 7.2 pmole mg$^{-1}$ hr$^{-1}$. † Control brains; they had been maintained at — 80°C for up to 2 years prior to assay. ‡ Samples from patient's brain were frozen in liquid nitrogen immediately after autopsy and assayed 1 month later.

ing effects of deficient ganglioside synthesis during development emphasize their important role in the development of the central nervous system and visceral organs. To avoid confusion with the classical sphingolipidoses, which are caused by deficiencies in catabolic enzymes, we propose the name anabolic sphingolipidosis-type $G_{M3}$ to describe this new disease. It will be interesting to see whether other severe diseases with similar clinical signs will fall into this potentially new class of anabolic sphingolipidoses.

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## Polarizing Fucoid Eggs Drive a Calcium Current through Themselves

Abstract. *Calcium ions enter the prospective growth pole of polarizing* Pelvetia *eggs faster than the opposite pole and leave this antipode faster than the growth pole. The resultant calcium current is greatest when first measured at 6 hours after fertilization and decreases as the time of final commitment to growth in a particular direction approaches.*

A unifying feature of the eggs of both plants and animals is the existence of a pattern which foreshadows the organization of the adult. The mechanisms by which these patterns are formed and maintained are not understood. The eggs of the marine brown alga *Fucus* and its close relative *Pelvetia* are convenient for studying this problem since they, unlike almost all other eggs, have no pattern at the time of fertilization. Some hours later they develop an axis, which is manifested by a bulging of the surface about 10 to 12 hours after fertilization. This growing region eventually becomes separated by a cell wall, and the cell thus formed gives rise to the rhizoid or holdfast of the adult plant while the other cell becomes the thallus. The direction in which the axis will form within the egg can be controlled by a variety of external vectors, including unilateral light, which causes the rhizoid to appear on the shaded side.

A clue to the mechanism that leads the fucoid egg from its initial unpolarized state to the grossly polarized embryo came with the discovery that these eggs drive an electrical current through themselves (*1*, *2*). It was suggested that the cytoplasmic field produced by the current might act electrophoretically to move charged entities to the growing region and thus be an essential link in the polarization process. However, the magnitude of the transcytoplasmic electric field depends strongly on which ions are carrying the current (*3*). Ions such as calcium that tend to be strongly bound to cellular components would produce a much larger field than those that are not. We therefore wanted to know if a calcium current was a significant part of the total current through the fucoid egg.

In order to answer this question, we developed a method for measuring the calcium ion fluxes across either half of the membrane of a developing *Pelvetia* egg. This was accomplished by using a 25-$\mu$m-thick nickel screen perforated with many closely spaced, round holes, each 75 $\mu$m in diameter (*4*). Each hole of such a screen was filled with an egg 90 $\mu$m in diameter (see cover photograph) and the egg-filled screen was then continuously exposed to 1000 foot candles ($\sim$ 11,000 lu/m$^2$) of unilateral white light. All of the rhizoids later formed on the screen's shaded side, away from the light. The fluxes of $^{45}Ca^{2+}$ into (or out of) the lighted and shaded sides of egg-bearing screens were compared to obtain the desired ratios of calcium fluxes into (or out of) the tentative rhizoid and thallus ends of the eggs.

All of the 25,000 holes in each screen were filled with eggs by a simple procedure. The screen was placed as a 25-mm-wide diaphragm in a Plexiglas chamber filled with seawater (Fig. 1a). The holes are funnel-shaped, and each screen was placed with its funnels inverted, that is, with its wide apertures downward (Fig. 1b). A suspension of recently fertilized *Pelvetia* eggs, obtained as described elsewhere (*5*), was injected into the lower part of the chamber and thus forced upward through the screen. After every hole was plugged with an egg, the eggs were

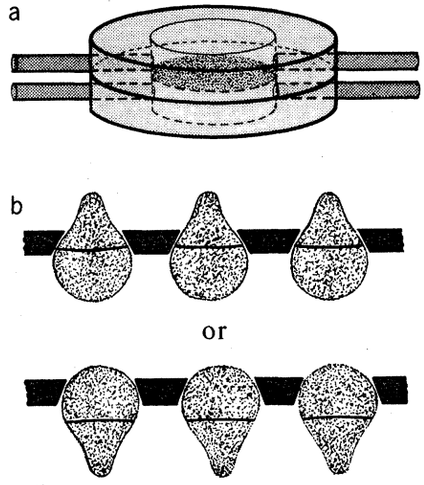


Fig. 1. (a) Chamber of the type used for flux measurements. The nickel screen is positioned as a diaphragm between the upper and lower halves of the chamber. (b) Sketch of a cross-sectional view of two egg-filled screens. The eggs shown here have begun to grow, but the measurements reported in the text were done before growth began so areal corrections were unnecessary.