side with the mobility of G_{M1}, indicating a G_{D1a} structure. This ganglioside does not inhibit TSH binding in the concentrations tested, in agreement with the previous observation that G_{D1a} from brain is a poor inhibitor (*1*). Thus, the ganglioside present in the highest concentration in bovine thyroid shows little ability to inhibit TSH binding.

A multiplicity of gangliosides is present on thyroid plasma membranes (*15*). These gangliosides vary in their ability to inhibit TSH binding to thyroid membranes. The most potent inhibitor (fraction 20) contains at least two sialic acid residues and some structure still undescribed. It constitutes 0.015 percent of the total thyroid gangliosides, or approximately 10,000 molecules per thyroid cell (*16*), a figure similar to that postulated for the number of cholera toxin receptors on fat cells (*4*). The predominant ganglioside of the bovine thyroid (tentatively G_{D1a}) is a poor inhibitor of TSH binding. In our study, those thyroid gangliosides that were the best inhibitors are present in the lowest concentrations, while those gangliosides showing minimal ligand interaction were present in higher concentration.

An earlier study indicated that TSH can bind to a glycoprotein membrane component (*17*). Gangliosides with a high affinity for TSH may act alone or in concert with this glycoprotein in receiving and transmitting the hormonal message to the thyroid target cell. It has been shown (*18*) that the disialyl residue present in gangliosides with a high affinity for TSH (G_{D1b}, G_{T1}, and possibly fraction 20) also is present in some glycoproteins. Gangliosides and glycoproteins may both contribute to the formation of a functional TSH receptor on the thyroid cell.

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15. Lagrou *et al.* (*14*) showed that gangliosides are concentrated in the plasma membrane fraction of bovine thyroid tissue. Also, the thyroid membrane preparations used in our study contain five times more LBSA per milligram of protein than crude homogenates (*1*).
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20. We thank S. Wollman for discussion concerning the number of cells in thyroid tissue.

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Deficiencies of Glucosamine-6-Sulfate or Galactosamine-6-Sulfate Sulfatases Are Responsible for Different Mucopolysaccharidoses

Abstract. *[1-^{3}H]Galactitol-6-sulfate,* N-*[1-^{3}H]acetylgalactosaminitol-6-sulfate,* N-*[1-^{3}H]acetylglucosaminitol-6-sulfate,* N-*acetylglucosamine-6-sulfate, and 6-sulfated tetrasaccharides from chondroitin-6-sulfate have been used for the measurement of 6-sulfatase activity of extracts of normal skin fibroblasts and of fibroblasts cultured from patients with genetic mucopolysaccharidoses. With these substrates, extracts of fibroblasts derived from Morquio patients lack or have greatly reduced activities for galactitol-6-sulfate,* N-*acetylgalactosaminitol-6-sulfate, and 6-sulfated tetrasaccharides but have normal activity for* N-*acetylglucosamine-6-sulfate and its alditol; those derived from a patient with a newly discovered mucopolysaccharidosis have greatly reduced activity for* N-*acetylglucosamine-6-sulfate and its alditol but normal activity for galactitol-6-sulfate,* N-*acetylgalactosaminitol-6-sulfate, and the 6-sulfated tetrasaccharides. These findings demonstrate the existence of two different hexosamine-6-sulfate sulfatases, specific for the glucose or galactose configuration of their substrates. Their respective deficiencies, causing inability to degrade keratan sulfate and heparan sulfate in one case and keratan sulfate and chondroitin-6-sulfate in the other, are responsible for different clinical phenotypes.*

Morquio disease (mucopolysaccharidosis IV) (*1*) emerged as a separate entity from the various osteochondrodysplasias when it was demonstrated that the affected patients excrete in the urine excessive amounts of keratan sulfate and accumulate in cartilage keratan sulfate and chondroitin-6-sulfate (*2*).

In 1974 Matalon *et al.* (*3*) prepared polymeric $^{35}SO_4$-labeled chondroitin-4/6-sulfate in chick embryos; using this material, oligosaccharides derived from it, and purified 6-sulfated oligosaccharides as substrates, they demonstrated that a sulfatase present in extracts of normal skin fibroblasts was absent or greatly defective in extracts of fibroblasts obtained from Morquio patients (*4*).

In 1976 Singh *et al.* (*5*) assayed the enzyme *N*-acetylgalactosamine-6-sulfate sulfatase, measuring sulfate released from tetrasaccharides derived from purified chondroitin-6-sulfate of shark cartilage, and confirmed that the enzyme is

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Table 1. Activity of galactose-6-sulfate, *N*-acetylgalactosamine-6-sulfate, and *N*-acetylglucosamine-6-sulfate sulfatases of extracts of normal fibroblasts, Morquio fibroblasts, and fibroblasts of a patient (G.G.) with a newly described mucopolysaccharidosis. The results are expressed as nanomoles of product per hour per milligram of protein; values are means ± standard errors of the means, with the range given in parentheses. Abbreviations: *N*, number of individual lines tested; ND, not detectable.

Fibroblasts	Activity (nmole/hour mg)				
	[^{3}H]Galactitol	*N*-[^{3}H]Acetylgalactosaminitol	Chondroitin-6-sulfate tetrasaccharides	*N*-[^{3}H]Acetylglucosaminitol	*N*-Acetylglucosamine
Normal (N = 7)	1.5 ± 0.09 (1.1 – 1.6)	0.9 ± 0.05 (0.8 – 1.0)	19 ± 2.2 (13.5 – 24)	1.8 ± 0.08 (1.5 – 1.9)	23 ± 1.9 (17 – 29)
Morquio (N = 5)	0.23 ± 0.11 (0 – 0.37)	0.08 ± 0.04 (0 – 0.15)	ND	1.5 ± 0.08 (1.3 – 1.7)	22 ± 4.8 (14 – 30)
Patient (G.G.)	1.3	1.3	15.3; 15.5*	0.16; 0.2*	ND

*Performed on extracts of different cultures.

below detectable levels in homogenates of Morquio fibroblasts.

As a result of these studies, it was concluded that the enzyme defective in Morquio disease was a 6-sulfatase presumably active on *N*-acetylglucosamine-6-sulfate of keratan sulfate and on *N*-acetylgalactosamine-6-sulfate of chondroitin-6-sulfate (*3, 4*). However, it was not clear why such a deficiency would not affect the degradation of heparan sulfate since this compound also contains *N*-acetylglucosamine-6-sulfate residues.

The study of a patient affected by an unusual mucopolysaccharidosis has provided evidence for the existence of two different hexosamine-6-sulfate sulfatases, specific respectively for *N*-acetylgalactosamine-6-sulfate and *N*-acetylglucosamine-6-sulfate. While a deficiency of the first enzyme is responsible for Morquio disease and for inadequate degradation of keratan sulfate and chondroitin-6-sulfate, a deficiency of the second enzyme is responsible for a novel disease in which the degradation of heparan sulfate and keratan sulfate are impaired.

The patient examined, G. G., who will be described in detail elsewhere (*6*), is a 5-year-old male with retarded growth, retarded intellect, a hypoplastic odontoid process of the atlas, mild osteochondrodystrophy, hepatomegaly, excessive and coarse hair, and excessive mucopolysaccharidurea consisting essentially of keratan sulfate and heparan sulfate. Cultured skin fibroblasts from the patient, unlike those derived from Morquio patients, showed inadequate degradation of intracellular, $^{35}SO_4$-labeled glycosaminoglycans. The various enzymes responsible for the known mucopolysaccharidoses, when measured on extracts of his fibroblasts or leukocytes or on his serum, were found to be normal.

Normal and mutant cultured skin fibroblasts (*7*) from one or more 75-cm^2 plastic plates were removed by trypsinization and collected by centrifugation (*8*). The pellets were suspended in 1 ml of 0.15*M* NaCl and sonicated for 20 seconds to disrupt the cells. The suspensions were dialyzed overnight against 0.15*M* NaCl and the retentates were cleared by centrifugation at 20,000*g* for 10 minutes at 5°C. The protein content of the clear supernatants was measured (*9*) and portions of the extracts were used for enzyme assays.

Galactose-6-sulfate, *N*-acetylgalactosamine-6-sulfate, and *N*-acetylglucosamine-6-sulfate were prepared with chlorosulfonic acid by the method of Suzuki and Strominger (*10*). The barium salts were converted to potassium salts; the preparations were desalted by gel filtration on Biogel P-2 columns (2 by 180 cm, 50 to 100 mesh) and analyzed by the chromatographic method of Dietrich *et al.* (*11*), using their solvent B. The bands corresponding to the 6-sulfated sugars were eluted with water, concentrated, and analyzed by infrared spectroscopy. All preparations showed a major band at 1235 cm^{-1} (S–O stretching) and minor ones at 775, 825, and 995 to 1000 cm^{-1}, indicative of sulfate in the 6 position. No evidence of sulfate in the 4 position was obtained. The three 6-sulfated monosaccharides were reduced with sodium borotritide as described by Horton and Philips (*12*) and the specific activity of each ^{3}H-labeled hexitol was calculated as counts per minute per nanomole (cpm/nmole) of sulfate (*13*). Tetrasaccharides from chondroitin-6-sulfate were prepared as described previously (*5*).

A 50-μl portion of fibroblast extract (100 to 150 μg of protein) in saline solution plus 10 μl of 0.6*M* acetate buffer of *p*H 4.8 (containing 5 percent bovine serum albumin) were added in plastic centrifuge tubes to 40 μl of water containing one of the substrates as follows: 100 nmole of galactitol-6-sulfate (2700 cpm/nmole), 150 nmole of *N*-acetylgalactosaminitol-6-sulfate (1200 cpm/nmole), or 180 nmole of *N*-acetylglucosaminitol-6-sulfate (1200 cpm/nmole). Control tubes were prepared as follows: some had all the ingredients less the substrate; others had the substrate alone. All the tubes were incubated at 37°C for 5 hours; thereafter the two types of control tubes were mixed, and all the tubes were immersed for 2 minutes in boiling water and then centrifuged for 1 minute in a microcentrifuge at 10,000*g*. Each clear supernatant was aspirated, added to 0.8 ml of water, and applied to a 5 by 20 mm column of Dowex 1 × 8 (200 to 400 mesh, Cl^- form) packed in water. Because of their anionic charge the substrates are retained in the columns, while the desulfated products are not. Each water effluent plus 0.3 ml of water wash were collected directly into counting vials. After the addition of 10 ml of Aquasol (New England Nuclear), the vials were cooled and counted. The difference in counting rate between each control tube (which contained less than 1 percent of the radioactivity of the substrate employed) and the corresponding tube incubated with the fibroblast extract was used to calculate the nanomoles of products obtained in 1 hour per milligram of protein. In addition, *N*-acetylglucosamine-6-sulfate sulfatase and *N*-acetylgalactosamine-6-sulfate sulfatase were measured using either 500 nmole of nonradioactive *N*-acetyglucosamine-6-sulfate or 500 nmole of sulfated tetrasaccharides from chondroitin-6-sulfate and incubation periods of 24 hours. In both cases, the activity was expressed as nanomoles of inorganic sulfate released per hour per milligram of protein (*13*). Table 1 shows the various enzyme activities found in the normal and mutant fibroblast extracts tested.

While extracts of normal fibroblasts are active on all the substrates used, those derived from Morquio patients are defective when tested with galactitol-6-sulfate, *N*-acetylgalactosaminitol-6-sulfate, or 6-sulfated tetrasaccharides from chondroitin-6-sulfate; however, they have normal activities when tested with *N*-acetylglucosamine-6-sulfate or its alditol. Conversely, extracts of fibroblasts obtained from patient G.G. have normal activities when tested with galactitol-6-sulfate, *N*-acetylgalactosaminitol-6-sulfate, and 6-sulfated tetrasaccharides from chondroitin-6-sulfate; however, they are defective with *N*-acetylglucosamine-6-sulfate or its alditol as sub-

strates. The higher activities obtained with the nonradioactive substrates are probably due to the higher concentrations of substrates used (about twice the Michaelis constant values found for the crude enzymes) or to a higher enzyme affinity for the nonreduced substrates in the pyranose ring conformation.

No inhibition of normal sulfatase activities was detected when extracts of normal fibroblasts, mixed in various proportions with extracts of fibroblasts from Morquio patients or from patient G.G., were incubated with the various substrates. With all substrates used, the enzymatic activities of normal fibroblast extracts were linear with respect to increases in time of incubation or protein concentration. The *p*H optimum for the sulfatase activities measured with the various substrates was 4.8, in agreement with the value found previously with chondroitin-6-sulfate tetrasaccharides (*5*). Half-maximal activities were observed at *p*H 3.7 and 5.6. Despite these similarities between the two postulated 6-sulfatases, their different sensitivities to lyophilization, concentration of ammonium sulfate or various ions, and distribution in various organs (*14*) strongly suggest that they represent different proteins.

These findings, obtained either with nonradioactive substrates (which occur in keratan sulfate, heparan sulfate, and chondroitin-6-sulfate) or with more convenient, radioactive derivatives of the substrates, support the suggestion (*3–5*) that the deficient enzyme in classical Morquio disease is a 6-sulfatase specific for substrates with the galactose configuration (*N*-acetylgalactosamine-6-sulfate and galactose-6-sulfate). Moreover, they demonstrate the existence of a separate 6-sulfatase, specific for substrates with the glucose configuration (*N*-acetylglucosamine-6-sulfate), whose deficiency is responsible for a novel mucopolysaccharidosis which affects the degradation of keratan sulfate and heparan sulfate and has clinical and biochemical features which may be considered a composite of those of Morquio and Sanfilippo phenotypes.

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Somatically Evoked Magnetic Fields of the Human Brain

Abstract. *The human brain is found to produce a magnetic field near the scalp which varies in synchrony with periodic electrical stimulation applied to a finger. Use of a highly sensitive superconducting quantum interference device as a magnetic field detector reveals that the brain's field is sharply localized over the primary projection area of the sensory cortex contralateral to the digit being stimulated. The phase of the response at the stimulus frequency varies monotonically with the repetition rate and at intermediate frequencies yields a latency of approximately 70 milliseconds for cortical response.*

We report here the detection of magnetic fields associated with the flow of electric current in the brain in response to electrical stimulation of the fingers. Weak magnetic fields resulting from visual stimulation have previously been detected outside the scalp (*1–4*). In contrast to the diffuse nature of the visually evoked potential (VEP), which is conventionally measured with scalp electrodes, the visually evoked field (VEF) is located over the visual cortex (*2*). This is to be expected as the electric currents giving rise to the VEF flow within the visual projection areas of the brain, while accompanying weaker currents in the skin remain undetected. The VEF has proved to be a significant indicator of brain function since its latency is directly correlated with the reaction time of a subject when the spatial frequency of a stimulus is varied (*5*, *6*). The confined location of the VEF and its correlation with a measure of human performance prompted us to search for neuromagnetic responses evoked by other stimuli. The neuromagnetic response evoked by electrical stimulation of individual fingers of the hand—the somatically evoked field (SEF)—is similarly found to be well localized over the primary projection area, in this case on the contralateral hemisphere in the region SI for the stimulated digit. The observed features of the SEF indicate that the neuromagnetic techniques can provide unique advantages for medical research.

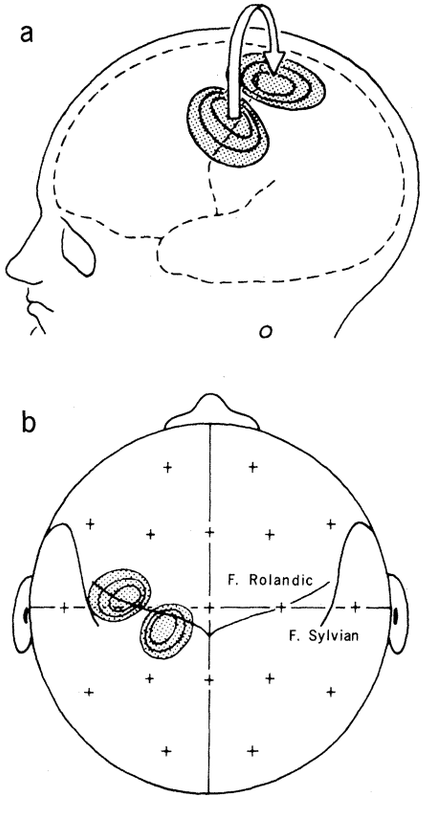

Fig. 1. (a) Neuromagnetic field patterns on the left hemisphere for an electrical stimulus at 13 hertz applied to the little finger of the right hand. Contours of equal magnetic flux indicate the relative amplitude of response for 0.9, 0.7, and 0.5 of the maximum response at the stimulus frequency. (b) The same pattern drawn on the conventional 10–20 electrode map. Abbreviation: *F.*, fissure.

A split-ring electrode, fashioned from a rubber grommet and two stainless steel

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