tracellular enzymes did not appear to be involved, for heated (80°C, 1 hour) and unheated spent acid mediums were equally effective in solubilizing basalt A.

Many of the rocks susceptible to fungal attack showed alterations in their infrared absorption spectra in the Si-O stretching vibration region. The changes generally observed were either the appearance of an absorption band in the region between 1060 and 1100 cm^{-1}, or intensification of a band in this region relative to neighboring bands at lower frequencies (Fig. 1). Basalt A showed a shift in absorption from a lower to a higher frequency; this was most pronounced in the difference spectrum (sterile rock versus inoculated rock). Dunite DTS-1 showed the appearance of a new band at 1080 cm^{-1} and a general diminution in intensity of the other bands. Intensifications similar to the latter were observed in basalts D-100043 and BCR-1 and in quartzite D-100316 (not illustrated in Fig. 1). None of the granites or other rocks, whether or not susceptible to fungal attack, showed altered infrared absorption spectra in this region.

One explanation for the spectral changes observed is that fungal attack disrupted the ordered crystalline structures of one or more silicate minerals with subsequent production of amorphous silicates with major absorption bands between 1060 and 1100 cm^{-1}. Figure 1 shows that amorphous silicates, such as fused quartz and silica gel, have major absorption bands in this region. On the other hand, it is equally plausible that silicate minerals absorbing between 1060 and 1100 cm^{-1} were already present but were masked or made to appear less intense by overlapping broad intense neighboring bands of other silicates. Complete or partial destruction of the latter would eliminate or diminish the intensity of the corresponding absorption bands and would unmask or appear to intensify the 1060 to 1100 cm^{-1} silicate bands.

Additional experiments with basalt A revealed that spent acid medium as well as equivalent concentrations of citric acid, oxalic acid, HCl, H_2SO_4, or H_3PO_4 solubilized comparable amounts of Si, Al, Fe, and Mg and caused similar alterations in the infrared absorption spectra. Thus, solubilization of basalt A and the concomitant spectral changes were not unique to biogenic acid but were the result of acid attack in general. A more detailed description of these and other experiments will be published elsewhere.

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7. Gas chromatography of the trimethyl esters of authentic citric acid and the unknown gave identical retention times and peak shapes, as did a mixture of the two; their mass spectrometric fragmentation patterns were also identical.
8. We thank R. Janda for guidance in the field, R. J. P. Lyon for valuable discussions, Dorothy I. Fennell for identifying *Penicillium simplicissimum* at the species level, and Patricia J. Kirk for technical assistance.

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Enzyme Replacement in Fabry's Disease, an Inborn Error of Metabolism

Abstract. *Two patients with Fabry's disease were infused with normal plasma to provide active enzyme (ceramide trihexosidase) for hydrolysis of the plasma substrate, galactosylgalactosylglucosylceramide. Maximum ceramide trihexosidase activity occurred 6 hours after infusion of the plasma, attaining a level approximately 150 percent of that in normal plasma; enzymatic activity was detectable for 7 days. The amount of accumulated substrate in the plasma of these recipients decreased about 50 percent on day 10 after infusion. Thus, periodic replacement of ceramide trihexosidase activity in the plasma of patients with Fabry's disease might lead to consistently lower amounts of substrate in the plasma and a decrease in its rate of accumulation in tissues.*

Fabry's disease is an inborn error of glycosphingolipid catabolism transmitted by an X-linked gene. It is characterized biochemically by the systemic accumulation of galactosylgalactosylglucosylceramide (gal-gal-glc-cer) (*1–3*) and by the deficiency of ceramide trihexosidase (galactosylgalactosylglucosylceramide : galactosyl hydrolase) in the tissues (*4*) and plasma (*5*) of hemizygous male patients. Increased amounts of gal-gal-glc-cer also occur in plasma (*2*), urinary sediment (*3, 6*), and cultured skin fibroblasts (*7*) from patients with this disease. It has been assumed that a major source of the accumulated glycosphingolipid is from the metabolism of globoside [2-acetamido-2-deoxygalactosyl-(β1 → 3)-galactosyl-(β1 → 4)-galactosyl-(β1 → 4)-glucosylceramide (*8*)] which occurs in the erythrocyte membrane and in lesser amounts in other tissues. This possibility is supported by studies of gal-gal-glc-cer, which accumulates in kidney, showing that the glycosidic linkages have the β configuration, that is, galactosyl-(β1 → 4)-galactosyl-(β1 → 4)-glucosyl-(β1 → 1′)-ceramide (*9*). This glycosphingolipid is accompanied by abnormal amounts of an incompletely characterized galactosyl-(1 → 4)-galactosyl-(1 → 1′)-ceramide in the kidney and pancreas, but not other organs (*1*).

The ceramide trihexosidase of normal plasma has greatest activity at *p*H

Table 1. Ceramide trihexosidase activity in plasma from normal volunteers and patients with various inborn errors of glycosphingolipid metabolism.

Disease	N	Age (years)	Sex	Ceramide trihexosidase activity*	
				pH 7.2	pH 5.4
None	8	20–45	M	15.0–15.8	7.9– 8.1
	8	18–25	F	12.9–22.0	6.2–10.4
Metachromatic leukodystrophy	1	10	M	13.0	5.5
Metachromatic leukodystrophy	1	8	F	14.3	8.4
Gaucher's (adult type)	1	30	M	15.3	7.6
Tay-Sachs	1	2	F	22.0	9.2
Fabry's (hemizygote)	8	4–60	M	<0.7	<0.7
Fabry's (heterozygote)	5	16–45	F	<0.7	4.0– 6.2

* Nanomoles of galactose liberated per hour per milliliter of plasma, as determined by the standard assay with galactosyl-(β1→4)-galactosyl-(β1→4)-glucosyl-(β1→1')-ceramide as substrate (*5*). The values represent ranges for *N* subjects.

7.2 and a second optimum at *p*H 5.4 (*5*). In plasma from patients with other glycosphingolipidoses such as Gaucher's disease, Tay-Sachs disease, and metachromatic leukodystrophy the enzymatic activity at both *p*H optima was normal (Table 1). In contrast, no detectable enzymatic activity was found at either *p*H optimum in hemizygotes with Fabry's disease (*5*), whereas heterozygotes exhibited detectable activity at *p*H 5.4 but not at *p*H 7.2 (Table 1).

Replacement of deficient ceramide trihexosidase activity in the plasma of patients with Fabry's disease with active enzyme might produce a decrease in the amount of the substrate, gal-gal-glc-cer, in the plasma and a lower rate of accumulation of this glycosphingolipid in tissues. The pure enzyme is not yet available for experiments with patients. Therefore, we have, as an alternative, infused two hemizygous male patients with normal human plasma.

Blood from each patient was assayed periodically for ceramide trihexosidase activity and for the concentration of gal-gal-glc-cer for 10 days after plasma infusion. Enzymatic activity was measured by a previously described method using galactose dehydrogenase for an end-point assay of free galactose liberated from the substrate, gal-gal-glc-cer (*5*). The amounts of gal-gal-glc-cer in plasma were determined (*10*), and the variation in triplicate analyses of gal-gal-glc-cer in control plasma was less than 5 percent; our values were not corrected for manipulative losses (*10*).

The hemizygous patients selected for this investigation had the characteristic clinical symptoms of Fabry's disease, as well as consistently high concentrations of gal-gal-glc-cer in the plasma (*2*) and urinary sediment (*3*), which chemically confirmed the clinical diagnosis. Ceramide trihexosidase activity at *p*H 7.2 and 5.4 was absent from the plasma of both patients (*5*).

Plasma for infusion was obtained by plasmapheresis of freshly drawn, heparinized blood from cross-matched normal donors whose previously assayed plasma had normal concentrations of ceramide trihexosidase activity. Prior to infusion, enzymatic activity in the normal plasma had decreased to one-fourth to one-half that in plasma from freshly drawn blood due to routine handling procedures (*11*). Plasma infusion in the recipients was completed within 30 minutes after venesection. A 17-year-old hemizygote [D.L., 115 pounds (1 kilogram = 2.2 pounds)] received 550 ml [2145 units (*12*)], and a 31-year-old hemizygote (A.G., 110 pounds) received 600 ml (4680 units).

The results of analyses for ceramide trihexosidase activity in the plasma of the recipients are shown in Fig. 1. Unexpectedly, the enzymatic activity increased beyond that anticipated. Maximum activity, which occurred at 6 hours after infusion in both patients, was 28.7 (A.G.) and 20.8 (D.L.) unit/ml. These values were 22- and 35-fold greater, respectively, than would be predicted from the volume and enzymatic activity of the infused plasma and were about 150 percent of the activity in normal plasma. The enzymatic activity decreased rapidly from 6 to 12 hours and then slowly until activity could no longer be detected after 7 days. Similar results have been obtained with a third hemizygote now being studied.

The concentrations of gal-gal-glc-cer in the plasma of each recipient decreased at a time just after the maximum activity of the peak of ceramide trihexosidase and then increased beyond initial levels coincident with the rapid decline in enzymatic activity (Fig. 1). From about 20 hours after infusion, there was a gradual decline in the concentration of gal-gal-glc-cer in the plasma and, after 10 days, it had decreased in both patients to about 50 percent of the initial concentrations (12.1 → 5.9 nmole/ml in A.G. and 7.7 → 4.4 nmole/ml in D.L.). The concentration of gal-gal-glc-cer at 30 days after infusion was up slightly to 6.3 nmole per milliliter of plasma in patient A.G.

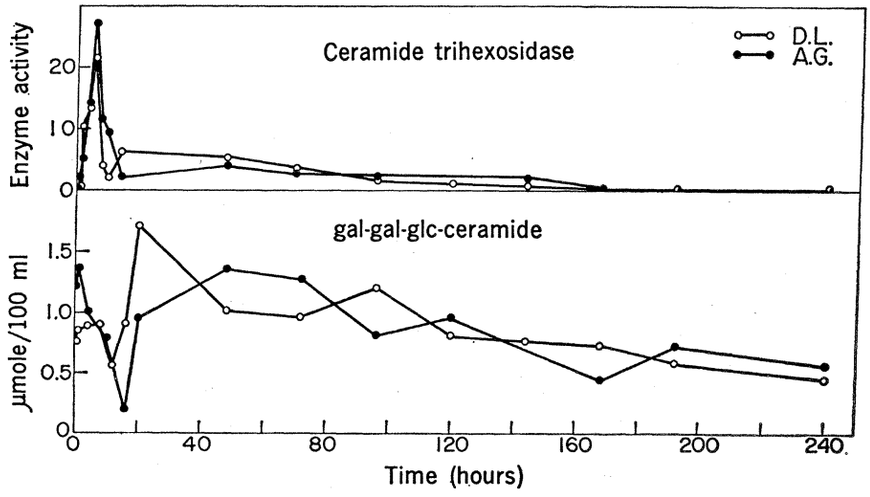

Fig. 1. (Top) Plasma ceramide trihexosidase activity in two hemizygous male patients with Fabry's disease after infusion of normal plasma (t_0 on the graph is at the end of the infusion). Enzymatic activity is expressed as nanomoles of galactose released from galactosyl-(β1→4)-galactosyl-(β1→4)-glucosyl-(β1→1')-ceramide per hour per milliliter of plasma at *p*H 7.2. (Bottom) Galactosylgalactosylglucosylceramide in plasma during the same period as above in the same patients after infusion of normal plasma. In a previous study (*2*) the mean value and average deviation of this glycosphingolipid in 15 samples of plasma from patient A.G., collected over a period of 4 months, was 0.76 ± 0.13 μmole/100 ml.

The correlation between increasing enzymatic activity followed by decreasing amounts of substrate during the first 16 hours after infusion is consistent with the expectation that the infused enzyme would initiate catabolism of the substrate in the plasma. The steadily decreasing concentration of gal-gal-glc-cer in the period after the first day correlates with the presence of low levels of ceramide trihexosidase in the plasma of both recipients throughout this period.

The kinetics of the decay of enzymatic activity suggest that at least two mechanisms are involved. The slow rate of decrease in the period from 1 to 7 days probably represents normal turnover of plasma enzyme, but the fast rate of decrease in the period from 6 to 12 hours is more difficult to explain. Possible incorporation of active enzyme into the tissues or adsorption onto the blood vessel walls has not been ruled out. It has already been reported that prolonged intravenous infusions of arylsulfatase A to patients with metachromatic leukodystrophy and of a crude glucosidase from *Aspergillus niger* to patients with type 2 glycogenosis leads to measurable enzymatic activity in hepatic tissue obtained by biopsy (*13*).

The unexpected enhancement of ceramide trihexosidase activity in the period from 0 to 6 hours is also of interest, and we are unaware of any published mechanisms which would uniquely account for it. However, attention might be directed to an analogous phenomenon in patients with von Willebrand's disease, a syndrome in which there is an inherited deficiency of factor VIII or antihemophilic factor. In these patients, factor VIII activity was enhanced eightfold after infusion with plasma obtained from normal donors or even from patients with factor VIII deficiency. Factor VIII activity reached a maximum about 24 hours after infusion (*14*).

We have sought to determine whether the enhancement of enzymatic activity in patients with Fabry's disease might be an in vitro phenomenon. Prior incubation at 37°C of equal volumes of plasma from a donor and a hemizygote did not increase the total enzymatic activity at *p*H 7.2. This suggests that the enhancement of activity observed in the patients is more likely to be an in vivo effect. A similar conclusion has been reached to account for the enhancement of factor VIII activity after normal plasma infusions in patients with von Willebrand's disease (*14*).

To determine whether normal plasma contained an activator, plasma from blood drawn into acid citrate-dextrose was frozen to inactivate the ceramide trihexosidase (*5*) and was subsequently infused into patient A.G. There was no detectable enzymatic activity in the recipient's plasma during the first 8 hours after infusion. The presence of an activator that is unstable upon freezing was not ruled out. Heparin alone in an amount twice as great as that used in the plasma infusions caused no detectable production of enzymatic activity in the recipient.

These findings provide an experimental basis for the hypothesis that enzyme replacement by plasma infusion will be a means of therapy for this glycosphingolipidosis. The proof of efficacy must rest on the results of clinical tests after a prolonged period of intermittent plasma infusions.

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Cadmium Toxicity Decreased by Dietary Ascorbic Acid Supplements

Abstract. *Feeding the environmental toxicant cadmium to young Japanese quail for 4 weeks produced growth retardation, severe anemia, low concentrations of iron in the liver, and high concentrations of cadmium in the liver. Dietary ascorbic acid supplements almost completely prevented the anemia and improved the growth rate but did not markedly alter concentrations of iron or cadmium in the liver.*

The hazards of industrial exposure to high concentrations of cadmium have been recognized for many years. More recently, attention has focused on cadmium intake from food, water, air, and cigarettes as a source of risk to the general population, primarily in the production of cardiovascular and respiratory diseases (*1*).

We have investigated the effect of a wide range of dietary components upon the toxic effects of cadmium in the young Japanese quail (*Coturnix coturnix japonica*). This bird has an exceptionally rapid growth rate and is very sensitive to dietary deficits and to toxic materials. We observed that dietary ascorbic acid supplements produce a marked protective effect on the anemia and a lesser protective effect on the depressed growth rate caused by cadmium.

Day-old birds of both sexes were fed an adequate purified soybean protein diet containing a total of 75 mg of zinc per kilogram and approximately 100 mg of iron per kilogram (*2*) for a period of 4 weeks. The basal diet was either fed alone or supplemented with cadmium, ascorbic acid, or cadmium plus ascorbic acid at the concentrations indicated in Table 1. Birds received