neutralization of endogenous RCP invariably terminated early pregnancies without curtailing implantation per se. The marginal weight gain in the RCP-immunized animals contrasts with the marked weight increases in the nonimmunized and ovomucoid-immunized animals during 18 days of gestation (Table 2) and reflects early embryonic loss in the RCP-immunized rats. Thus, it appears that physiological processes and endocrine functions vitally concerned with ovulation, fertilization, tubal transport, and subsequent blastocyst formation and implantation were not impaired by active immunization. After termination of early pregnancies the RCP-immunized animals resumed their 4-day estrous cycles and conceived normally when mated. Again there was no apparent interference with the process of implantation, but from day 9 of pregnancy the serum progesterone levels sharply declined indicating fetal death. This pattern of early interference with pregnancy could be demonstrated repeatedly as long as high antibody titers were maintained by booster injections of the antigen. If, however, the titer was allowed to wane with lapse of time (3 months after a booster injection), the animals carried their pregnancies to term and delivered pups with no detectable abnormality.

These findings not only demonstrate the reversibility of the effects of active immunization with RCP, but also imply that antibodies to the heterologous vitamin carrier, though capable of effectively neutralizing the endogenous RCP (and hence interfering with embryonic development), nonetheless fail to boost significantly the animals' immunological memory to render the process irreversible for prolonged periods. This is reminiscent of the situation pertaining to fertility regulation in subhuman primates (*18*) actively immunized with the β-subunit of human chorionic gonadotropin and in women administered the hormonal subunit coupled to tetanus toxoid (*19*).

These data indicate that the vitamin carrier protein is obligatory for transplacental riboflavin transport and supply to the developing fetus in higher animals. Similar proteins have been discovered in pregnant cows (*20*) and primates (*5*). It would thus be interesting to determine whether active immunization against the pregnancy-specific vitamin carrier could also terminate early pregnancy in primates.

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Abnormal Glutamate Metabolism in an Adult-Onset Degenerative Neurological Disorder

Abstract. *In patients with recessive, adult-onset olivopontocerebellar degeneration associated with a partial deficiency of glutamate dehydrogenase, the concentration of glutamate in plasma was significantly higher than that in controls. Plasma α-ketoglutarate was significantly lower. Oral administration of monosodium glutamate resulted in excessive accumulation of this amino acid in plasma and lack of increase in the ratio of plasma lactate to pyruvate in the glutamate dehydrogenase–deficient patients. Decreased glutamate catabolism may result in an excess of glutamate in the nervous system and cause neuronal degeneration.*

Degenerative neurological disorders, such as Huntington's chorea, Parkinson's disease, and spinocerebellar ataxia, are all characterized by selective, premature loss of nerve cells. The pathogenesis of this neuronal degeneration has been the subject of intense investigations, but remains unknown. In recent years considerable interest has been aroused by observations showing that certain neuroexcitatory amino acids, such as glutamate and its potent analogs kainic acid and ibotenic acid, are capable of producing selective nerve cell destruction while sparing other elements of nervous tissues (*1*). Furthermore, when these compounds are injected into certain brain areas of experimental animals, they produce morphological and biochemical alterations found in patients with degenerative neurological disorders (*1*).

Although these findings have raised the possibility that similar neurotoxic substances accumulate in human nervous tissues and cause neuronal degeneration, evidence for this has been lacking. In the present study we investigated patients with a genetic neurological disorder known as olivopontocerebellar atrophy (OPCA). The disease, a form of which is associated with a partial deficiency of glutamate dehydrogenase (GDH) (E.C. 1.4.1.3), affects adults and is characterized by progressive atrophy of areas of the brainstem, cerebellum, spinal cord, and substantia nigra and by ataxia, corticospinal deficits, dysarthria, dysphagia, and signs of parkinsonism (*2*). It is regarded as the classical structural neurological disorder linking the spinocerebellar degenerative conditions with the extrapyramidal diseases. We found decreased glutamate catabolism in patients with this condition. The accumulation of glutamate in the nervous tissue may well be the cause of the neuronal degeneration.

In previous studies, we showed that leukocytes and cultured skin fibroblasts from patients with a recessive form of OPCA have decreased GDH activity (*3*). The enzymatic deficiency was identified when several enzymes requiring nicotinamide adenine dinucleotide phosphate [NAD(P)] were evaluated in the fibroblasts. The rationale for these investigations was based on observations of the neurotoxic effects of the nicotinamide antagonist 3-acetylpyridine in the rat (*3*).

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Table 1. Enzyme activities in glutamate and α-ketoglutarate metabolism in homogenates of leukocytes from the OPCA patients (N = 7) and the controls (N = 14). Leukocyte pellets were isolated from 20 ml of venous blood (*3*); disrupted cells were homogenized in 10 m*M* tris (*p*H 7.4). Enzyme activities, expressed as micromoles of substrate utilized (or product formed) per milligram of protein per hour, were measured in whole homogenates. GDH activity with no additions or with 1 m*M* adenosine diphosphate, NADP–isocitrate dehydrogenase (E.C. 1.1.1.42), cytoplasmic aspartate aminotransferase (E.C. 2.6.1.1), or mitochondrial aspartate aminotransferase were determined as previously described (*3*). Alanine aminotransferase (E.C. 2.6.1.2) was measured by the method of Segal and Matsuzawa (*13*). Protein was determined by the method of Lowry *et al.* (*14*). Values are means ± standard deviations.

Group	GDH	GDH + ADP	NADP isocitrate dehydrogenase	Cytoplasmic aspartate amino-transferase	Mitochondrial aspartate amino-transferase	Alanine amino-transferase
Control subjects	0.41 ± 0.07	0.72 ± 0.11	1.29 ± 0.31	2.86 ± 0.64	0.99 ± 0.23	0.41 ± 0.04
Experimental subjects	0.19 ± 0.03*	0.35 ± 0.07*	1.29 ± 0.17	3.01 ± 0.73	0.98 ± 0.32	0.45 ± 0.11

*GDH activities (nonstimulated and ADP-activated) in leukocytes from experimental subjects differ significantly ($P < .001$) from activities in leukocytes from controls. The defect was specific for GDH, since the activities of other metabolic enzymes were the same in all the groups.

Additional studies of leukocytes from seven patients with recessive OPCA have corroborated data indicating a specific defect in GDH activity (Table 1). However, the relation between the biochemical defect and pathogenesis of the disease has remained obscure. GDH is thought to synthesize glutamate from α-ketoglutarate by ammonia fixation, with formation of oxidized NAD(P), or to deaminate glutamate to α-ketoglutarate, with production of reduced NAD(P) [NAD(P)H]. The enzyme is also thought to function in maintaining the mitochondrial NAD/NADH potentials, which in turn are in equilibrium with the cytoplasmic potentials, as reflected in the ratio of lactate to pyruvate (*4*). In an attempt to elucidate the pathophysiology of the GDH deficiency, we studied the metabolism of substrates of the enzyme-catalyzed reaction in patients with this enzymatic deficiency.

We obtained the informed consent of seven patients (from six families) with clinically diagnosed OPCA and decreased GDH activity in leukocytes (Table 1). Slowly progressive neurological dysfunction had been afflicting these patients after their fourth decade. Clinical characteristics were cerebellar ataxia, extrapyramidal manifestations (parkinsonism), corticospinal deficits, bulbar nerve dysfunction, some amyotrophy, and sphincter disturbances (*3*). Atrophy of the brainstem and cerebellum as well as some diffuse cerebral atrophy were demonstrated by computerized axial tomography of the head. Genetic data indicating that the disease in these patients was recessively inherited have been presented elsewhere (*3*). Five of the patients were ambulatory at the time of the study. They were all on a regular diet and their nutritional status was good.

There were two control groups. The first group, normal controls (N = 36), included only healthy adults. The second group, diseased controls (N = 20), included patients with various types of spinocerebellar degeneration not associated with GDH deficiency. None of the subjects was receiving any medication at the time of the study, and they all had normal blood chemistry and thyroid test results.

The subjects fasted overnight. Blood samples were obtained between 8 and 9 a.m. for the measurement of plasma amino acids, α-ketoglutarate, ammonia, lactate, and pyruvate. Drawn blood was immediately transferred to chilled (0° to 5°C), EDTA-containing tubes. The plasma was separated by centrifugation at 19,000*g* for about 10 minutes and then deproteinized by the addition of 2N perchloric acid (0.4N final concentration). The protein-free supernatant was separated by another centrifugation (19,000*g*) and then stored at −80°C until the biochemical analysis. Eighteen amino acids were measured with a Technicon TSM amino acid analyzer (*5*); great care was taken to prevent hydrolysis of glutamine to glutamate in the plasma extracts (*6*). Pyruvate, α-ketoglutarate, lactate, and ammonia concentrations were measured in neutralized plasma extracts by enzymatic and fluorometric techniques (*7*). In addition, two "loading" tests were carried out. In the first test, patients and controls, after fasting overnight, were given a standard meal containing 1 g of protein per kilogram of body weight. Protein can contain up to 11 percent glutamate (*8*). After 2 and 4 hours, blood samples were drawn for the measurement of amino acids in the plasma. In the second test, monosodium glutamate (60 mg/kg) dissolved in 100 to 150 ml of water was orally administered to the subjects after they fasted overnight. Blood samples were obtained after 30, 60, 90, and 120 minutes and were processed and analyzed as described above.

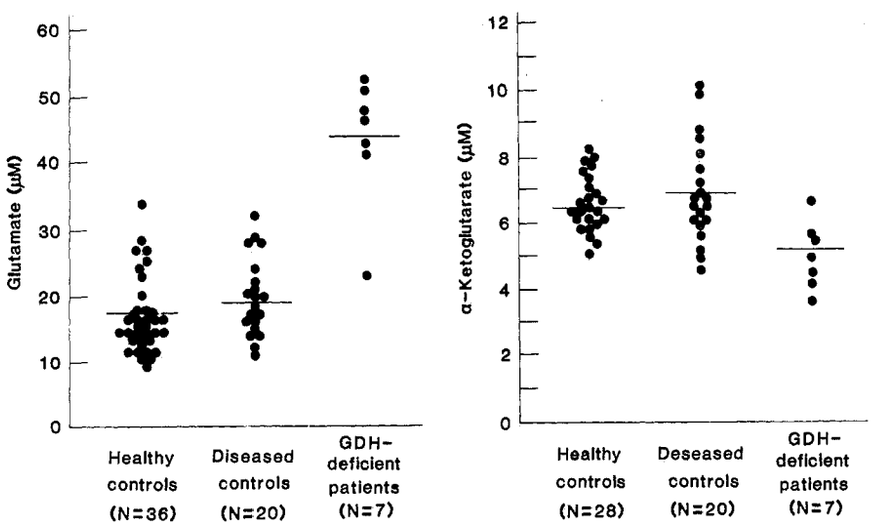

Fig. 1. Plasma glutamate and α-ketoglurate concentrations in GDH-deficient patients and in controls. Data points represent average levels for each individual (at least two blood samples were analyzed for each patient and for most diseased controls); lines indicate the mean value for each group.

The mean concentrations of plasma glutamate in the normal controls and diseased controls were 17.39 ± 6.38 and

19.90 ± 6.03 μmole/liter, respectively. Plasma glutamate in the patients with deficient GDH was significantly higher (43.12 ± 10.49 μmole/liter; $P < .0005$, Student's t-test) (Fig. 1). Six of the seven GDH-deficient patients had plasma glutamate concentrations that were more than 2 standard deviations higher than the control means. In contrast, the concentration of plasma α-ketoglutarate in the GDH-deficient patients was 21 percent less than that in the healthy controls ($P < .001$) and 26 percent less than that in the diseased controls ($P < .005$) (Fig. 1). There were no between-group differences in the concentrations of ammonia, aspartate, threonine, serine, asparagine, glutamine, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, ornithine, lysine, histidine, and arginine. At 2 and 4 hours after protein loading, glutamate and aspartate rose to significantly higher levels in the GDH-deficient patients than in the controls (Fig. 2A). Alanine also showed a small but significant increase after 4 hours. Similarly, ingestion of monosodium glutamate led to far greater elevations in plasma glutamate and aspartate in the experimental subjects than in the controls (Fig. 2B). However, alanine levels did not change significantly in any group. The lactate to pyruvate ratio increased significantly in the controls 90 and 120 minutes after monosodium glutamate ingestion (Fig. 2B). The ratio in patients with GDH deficiency did not change.

These results clearly show impaired catabolism (oxidative deamination) of glutamate in GDH-deficient OPCA patients, with resultant glutamic acidemia and reduction in the plasma pool of α-ketoglutarate. In addition, the protein loading test indicates an impaired rate of catabolism of exogenous (dietary) glutamate, with increased formation of aspartate and alanine by transamination. The glutamate loading test substantiated these findings by showing excessive accumulation of plasma glutamate, which also resulted in increased formation of aspartate through transamination. The lack of changes in plasma alanine after glutamate loading is in agreement with the findings that oral intake of monosodium glutamate does not result in increased plasma alanine and that ingestion of a protein hydrolyzate does (*8*).

Since significant increases in the lactate to pyruvate ratio occurred in controls after monosodium glutamate intake, it may be concluded that oxidative deamination of glutamate by GDH in these individuals produced enough reduced NADH to alter (increase) the ratio of NADH to NAD. The lack of such an increase in the GDH-deficient patients is direct evidence that the excessive increases in plasma glutamate in these patients were the result of a defect in the utilization of the oxidative deamination pathway.

Elevated concentrations of plasma glutamate have been reported to occur in patients with gout or uremia (*9*), and apparent increases may also be seen in conditions associated with elevated plasma glutamine. None of these conditions, however, was present in our patients.

Genetically determined defects in amino acid metabolism have been classically described to occur in infancy or early childhood as a result of almost complete enzymatic deficiencies transmitted as autosomal recessive traits. Although partial enzymatic defects in people heterozygous for these afflictions are considered to be without clinical manifestations, recent studies showed neurological dysfunction in adults (*10*). The pres-

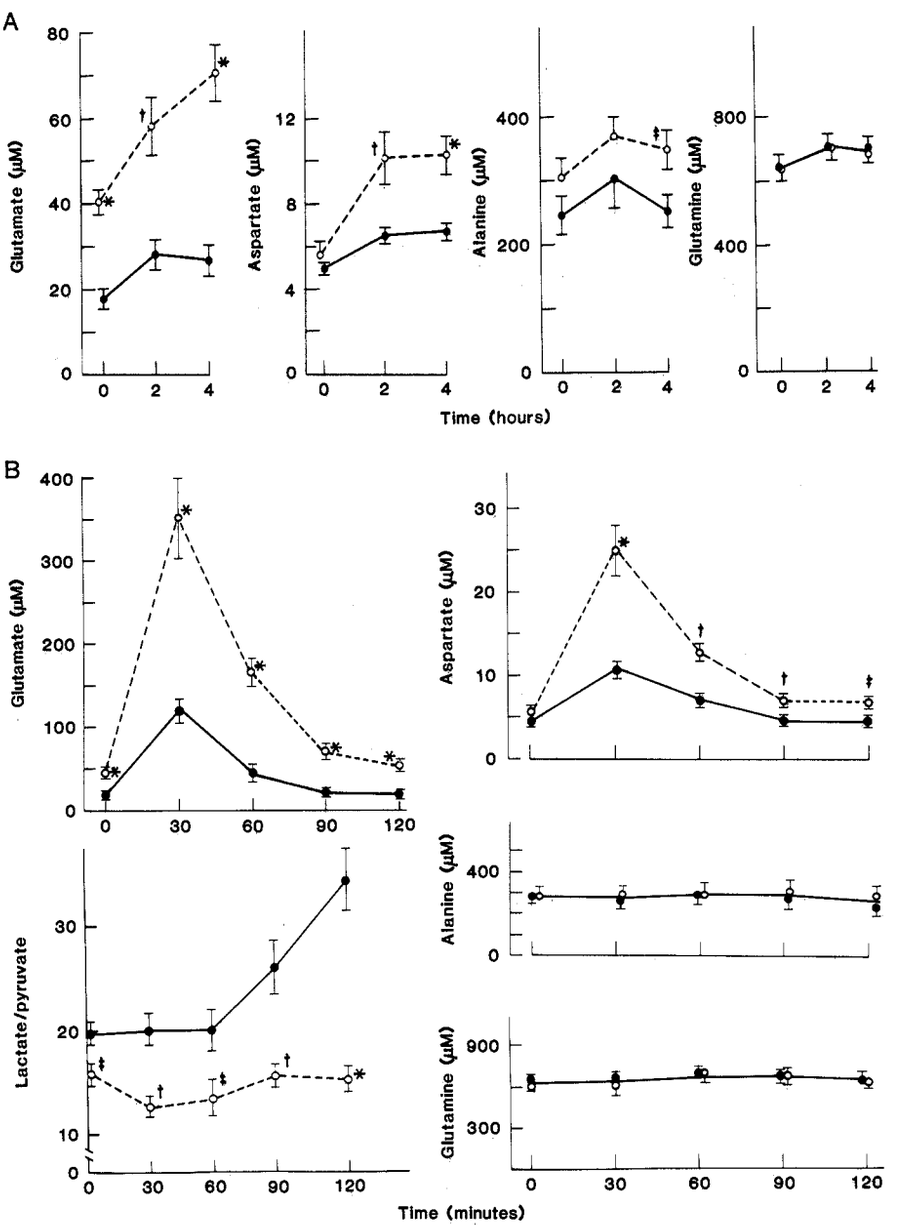

Fig. 2. Results for the protein and glutamate loading tests. (A) High protein test. After fasting overnight, patients with low GDH activity (○) ($N = 5$) and controls (●) ($N = 10$) consumed a meal containing 1 g of protein per kilogram of body weight. The control group included five healthy controls and five diseased controls. (B) Glutamate test. After fasting overnight, GDH-deficient patients (○) ($N = 6$) and controls (●) (five healthy and eight diseased) received monosodium glutamate (60 mg/kg, orally) dissolved in 100 to 150 ml of water. Blood samples were drawn at the times indicated and processed. Data points represent mean values, and bars 1 standard error. Symbols: (*) $P < .001$, (†) $P < .01$, (‡) $P < .05$.

ent study shows that an adult-onset, degenerative neurological disorder is associated with abnormal metabolism of the major excitatory amino acid (measured as glutamate) as a result of a partial enzymatic deficiency that probably is genetically determined. These findings raise the possibility that other genetically determined, adult-onset neurological diseases causing nervous system atrophy may also be related to partial enzymatic defects.

Since the metabolic defect is systemic, impaired glutamate metabolism probably occurs in the brain, the organ that has the highest concentrations of glutamate. The susceptibility of the nervous system to abnormal glutamate metabolism may explain why only this system is affected in OPCA patients. Although glutamate does not cross the blood-brain barrier in normal adults, it seems that decreased glutamate catabolism in the nervous tissue could, over many years, cause neuronal damage. GDH is unevenly distributed in the central nervous system (*11*), and it may be associated with the pool of glutamate that is released as a neurotransmitter at the nerve endings (*12*). Decreased catabolism of glutamate at the nerve terminals could result in an increased amount of the neurotransmitter at the synapses, leading to overexcitation and neuronal degeneration.

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Bronchoconstrictor Effects of Leukotriene C in Humans

Abstract. *Maximum expiratory flow rate at 30 percent of vital capacity above residual volume served as an index of airway obstruction in comparing the effects of leukotriene C and histamine administered by aerosol to five normal persons. Leukotriene C was 600 to 9500 times more potent than histamine on a molar basis in producing an equivalent decrement in the residual volume. The leukotriene C response was slow in onset and prolonged, reminiscent of the effects of aerosol allergen challenge in asthmatic allergic subjects.*

The airway constriction that occurs in the setting of immediate hypersensitivity reactions is thought to result from the effects of chemical mediators, released or generated as a consequence of the immunological response, on airway and airspace contractile tissues. Of the mediators identified to date, histamine is an unlikely candidate as a mediator of allergic bronchoconstriction (*1*), whereas a number of lines of evidence suggest that slow reacting substance of anaphylaxis (SRS-A) may be a major cause of this effect (*2*). Recently, it has been recognized that SRS-A is comprised of three leukotriene (LT) constituents—LTC, LTD, and LTE (*3–5*)—which have been shown to be potent mediators of bronchoconstriction in vitro for tissues of humans and lower mammals and in vivo in experimental animals (*6*). In a previous study (*7*) in which LTC was administered by aerosol to two normal persons, bronchoconstrictor activity was reported but the relative potency of LTC to other constrictor stimuli such as histamine was not determined; however, the cough response of the two subjects was so marked that the authors postulated an action of LTC on upper airway irritant receptors.

Five human volunteers (four males and one female, aged 21 to 36 years) without pulmonary disease, without a history of cigarette smoking, and with normal pulmonary mechanics gave informed consent to serve as experimental subjects. At the same time of day on two separate nonconsecutive days, dose-response data were obtained from the subjects as they inhaled aerosols generated from solutions of histamine or LTC with a DeVilbiss No. 42 nebulizer, with a dosimeter set at a constant delivery time (0.8 second) and pressure (20 pounds per square inch). This nebulizer delivers particles with an aerodynamic mass median diameter of 5 μm determined by impact analysis. The subjects inhaled to ~ 60 percent of vital capacity with a 2-second breath hold at the end of inspiration for ten breaths at all concentrations of both agents. Leukotriene C was prepared by total chemical synthesis (*4*); the histamine was obtained from Sigma. Both were diluted in phosphate-buffered saline (*p*H 7.40) within 30 minutes before administration.

Maximum expiratory air flow rate at 30 percent of control vital capacity above residual volume ($\dot{V}_{30}$) was measured from partial expiratory flow-volume (PEFV) maneuvers performed in triplicate immediately before and at defined times after each aerosol inhalation. This particular index was chosen because the full inflation of the lungs required to perform a maximum flow-volume maneuver could have reversed or attenuated the expected airway responses (*8*). All studies were conducted in an integrated-flow (pressure corrected) body plethysmograph according to standard techniques. The concentration of each agent in the nebulizer (expressed in terms of the chemical base) was increased in half-log increments until a 30 percent decrease in $\dot{V}_{30}$ occurred. Flow rates were measured 5 minutes after administration of histamine and 15 minutes after administration of LTC, corresponding to the time of the peak effect as had been determined from preliminary time-response experiments.

On day 3 of the experiment, the reproducibility of the response to a given dose from the cumulative dose-response curve and the detailed time course of the LTC effect was determined. Each subject was required to inhale a dose of LTC that had resulted in a greater than 30 percent decrease in $\dot{V}_{30}$ on a previous

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