Inborn errors of molybdenum metabolism: Combined deficiencies of sulfite oxidase and xanthine dehydrogenase in a patient lacking the molybdenum cofactor

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ABSTRACT A patient suffering from a combined deficiency of sulfite oxidase (sulfite dehydrogenase; sulfite:ferricytochrome c oxidoreductase, EC 1.8.2.1) and xanthine dehydrogenase (xanthine:NAD+ oxidoreductase, EC 1.2.1.37) is described. The patient displays severe neurological abnormalities, dislocated ocular lenses, and mental retardation. Urinary excretion of sulfite, thiosulfate, S-sulfocysteine, and xanthine oxidase activity was not defrayed, but the presence of the sulfite dehydrogenase (sulfite dehydrogenase, EC 1.2.1.37) was confirmed by high-voltage electrophoresis (40 V/cm; formic acid/acetic acid/water, 15:10:75 V/V; pH 1.18). S-Sulfocysteine can be distinguished from cysteic acid by its differing color with the ninhydrin/isatin staining reagent. Urinary purines and pyrimidines were analyzed by two-dimensional thin-layer chromatography (11) and by quantitative cation exchange column chromatography (12). The same thin-layer chromatographic technique was used for the analysis of urinary hydantoin 5-propionic acid after loading with L-histidine. Uric acid was measured with uricase (Leo Pharmaceutical, Ballerup, Denmark). Serum molybdenum concentrations were determined by neutron activation analysis (performed by I. Lombeck, University Children’s Hospital, Dusseldorf, West Germany).

Enzyme, Molybdenum, and Molybdenum Cofactor Assays. Hepatic sulfite oxidase and xanthine dehydrogenase activities and molybdenum content were determined as described (13), using a sample of tissue obtained by surgical biopsy. Xanthine dehydrogenase activity was also assayed by a fluorometric procedure using 2-amino-4-hydroxypteridine as substrate (14). Superoxide dismutase was assayed by Hosni Hassan of Duke University by its ability to inhibit xanthine oxidase-mediated reduction of cytochrome c (15). Both cyanide-sensitive (manganese-containing) and -insensitive (copper, zinc-containing) enzyme activities were measured. Monoamine oxidase activity was assayed with benzylamine (16) and alcohol dehydrogenase with ethanol (17) as respective substrates. Glutamate dehydrogenase was assayed as described (18).

Active molybdenum cofactor was assayed by its ability to reconstitute sulfite oxidase activity in a preparation of demolybdenal sulfite oxidase isolated from tungsten-treated rats (19) and by its ability to reconstitute nitrate reductase activity in extracts of the Neuspora mutant nit-1 (20).

MATERIALS AND METHODS

Analysis of Urinary Metabolites. Sulfite concentrations were estimated by using Merckquant Sulfit-test strips (E. Merck, Darmstadt, West Germany; also available from Scientific Products). Thiolsulfate was quantitated by using the method of Sorbo (9) with minor modifications, and sulfate was measured with a radiochemical procedure according to Miller et al. (10).

Amino acids were analyzed with a Technicon TSMI amino acid analyzer, using a standard procedure. For a quantitative recovery of acidic amino acids the sample cartridges were loaded with a strong anion-exchange resin (Technicon type S chromatobeads). The identities of S-sulfocysteine and taurine were confirmed by high-voltage electrophoresis (40 V/cm; formic acid/acetic acid/water, 15:10:75 V/V; pH 1.18). S-Sulfo- cysteine can be distinguished from cysteic acid by its differing color with the ninhydrin/isatin staining reagent. Urinary purines and pyrimidines were analyzed by two-dimensional thin-layer chromatography (11) and by quantitative cation exchange column chromatography (12). The same thin-layer chromatographic technique was used for the analysis of urinary hydantoin 5-propionic acid after loading with L-histidine. Uric acid was measured with uricase (Leo Pharmaceutical, Ballerup, Denmark). Serum molybdenum concentrations were determined by neutron activation analysis (performed by I. Lombeck, University Children’s Hospital, Dusseldorf, West Germany).

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Table 1. Characteristic urinary excretion of uric acid, oxypurines, and cysteine metabolites in a patient with a combined deficiency of xanthine dehydrogenase and sulfite oxidase

<table>
<thead>
<tr>
<th>Date</th>
<th>Conditions</th>
<th>Creatinine, mg/liter</th>
<th>Uric acid</th>
<th>Xanthine</th>
<th>Hypoxanthine</th>
<th>Sulfite</th>
<th>Thio-sulfate</th>
<th>S-Sulfocysteine</th>
<th>Taurine</th>
<th>Sulfate</th>
</tr>
</thead>
<tbody>
<tr>
<td>March 28, 1977</td>
<td>Normal diet</td>
<td>305</td>
<td>0.07</td>
<td>5.48</td>
<td>0.95</td>
<td>+</td>
<td>3.57</td>
<td>4.43</td>
<td>6.16</td>
<td>2.92</td>
</tr>
<tr>
<td></td>
<td>Sulfur-restricted diet</td>
<td>215</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>June 13, 1978</td>
<td>Restricted diet + oral Mo + NaHCO₃</td>
<td>260</td>
<td>0.16</td>
<td>4.19</td>
<td>0.85</td>
<td>+</td>
<td>2.23</td>
<td>2.43</td>
<td>1.15</td>
<td>3.30</td>
</tr>
<tr>
<td>May 28, 1978</td>
<td>Restricted diet + Mo + NaHCO₃ + allopurinol</td>
<td>330</td>
<td>&lt;0.03</td>
<td>3.70</td>
<td>0.71</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Cysteine loading*</td>
<td>I</td>
<td>125</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>6.38</td>
<td>3.20</td>
<td>4.56</td>
<td>4.48</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>110</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>4.79</td>
<td>3.09</td>
<td>4.91</td>
<td>2.50</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>275</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>4.42</td>
<td>2.80</td>
<td>3.60</td>
<td>2.87</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>865</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>5.85</td>
<td>3.04</td>
<td>5.82</td>
<td>3.67</td>
</tr>
<tr>
<td>Controls</td>
<td>4–10</td>
<td>0.06–0.25</td>
<td>0.02–0.07</td>
<td>Not detected</td>
<td>0.08</td>
<td>&lt;0.02</td>
<td>&lt;0.7</td>
<td>&gt;7.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Loading was 100 mg of L-cysteine per kg of body weight. Urine was collected in 6-hr portions, numbered I–IV.

Immunological Procedures. Antisera of high titer directed against human liver sulfite oxidase and rat liver xanthine dehydrogenase were raised in rabbits as described (7). Immunoglobulins were purified by ammonium sulfate fractionation (0–33%) and chromatography on DEAE-cellulose DE-52 (Whatman). Immunodiffusion plates were purchased from Vega Chemicals (Tucson, AZ). Radioimmunoassay of sulfite oxidase crossreacting material was performed by a procedure to be published elsewhere. Human liver sulfite oxidase was iodinated with 125I by using the chloramine-T procedure, and a double antibody method was used to separate bound radioactivity from free.

Fibroblast Studies. Fibroblasts were cultured from punch skin biopsy materials as described (21). Cells were lysed for analysis by sonication in 5 vol of 10 mM potassium phosphate buffer, pH 7.8. The lysates were centrifuged at 20,000 X g for 10 min. Samples of the supernatant fractions were used directly for radioimmunoassay or subjected to gel filtration for sulfite oxidase activity assay (21).

RESULTS

Clinical Summary. The patient has shown feeding difficulties almost from birth. On admission a small child (length and weight 10th percentile) was seen with an asymmetric skull and frontal bossing. Skull circumference was at the 50th percentile. Eye abnormalities included bilateral dislocated ocular lenses, enophthalmus, nystagmus, and a ring of Brushfield spots. Typical tonic-clonic seizures have always been present. Her electroencephalogram showed diffuse irregularities and on the pneumoecephalogram widened ventricles and periventricular atrophy were seen. At the age of 3 years the patient has not achieved any developmental milestones. She is bedridden, does not react to light or sounds, and is extremely hypertonic. Her length and weight are now at the 50th and 3rd percentiles, respectively.

The diagnosis of sulfite oxidase deficiency plus xanthine dehydrogenase deficiency was first suspected after the finding of abnormal metabolites during a multi-chromatographic screening of the patient's urine. These metabolites included sulfite, S-sulfocysteine, taurine, xanthine, hypoxanthine, and uric acid (see Table 1). The metabolic profile was completed by analyzing the urinary excretion of thiosulfate and sulfate (see Table 1).

Attempts at Treatment. In her first year of life the patient received normal feedings with respect to her age. After the diagnosis of sulfite oxidase deficiency had been made, a low-cysteine diet was prescribed, equivalent to 12 mg of cysteine per kg of body weight. In order to counteract a possible sulfate deficiency extra oral Na₂SO₄ (700 mg/day) was given. No side effects of this administration were observed. Because of the presence of xanthine gravel in the diapers, allopurinol medication was started (10 mg/kg per day) in an attempt to shift the xanthine-to-hypoxanthine ratio to the more soluble hypoxanthine. Because xanthine is more soluble at higher pH, the pa-

Table 2. Levels of molybdenum, molybdoenzymes, and molybdenum cofactor in liver tissue

<table>
<thead>
<tr>
<th>Subject</th>
<th>Molybdenum, µg/g</th>
<th>Xanthine dehydrogenase activity, units/g</th>
<th>Sulfite oxidase activity, units/g</th>
<th>Sulfite oxidase crossreacting material, units/g</th>
<th>Sulfite oxidase, units/g</th>
<th>Molybdenum cofactor, units/g</th>
<th>Sulfite oxidase</th>
<th>Nitrate reductase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>0.92</td>
<td>0.76</td>
<td>13.5</td>
<td></td>
<td>72</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>0.99</td>
<td>0.89</td>
<td>27.5</td>
<td></td>
<td>75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>0.38</td>
<td>0.46</td>
<td>13.5</td>
<td>14.9</td>
<td>55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child, 4 mo</td>
<td>0.40</td>
<td>0.44</td>
<td>13.4</td>
<td></td>
<td>35</td>
<td>312</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child, 6 mo</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.1</td>
<td></td>
<td>&lt;2</td>
<td>&lt;50</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Based on a specific activity of 918 units per mg of human sulfite oxidase.
tient was given 250 mg of NaHCO₃ four times daily. Finally a trial with oral supplements of molybdenum [up to 200 μg of Mo per day, given as (NH₄)₆MoO₄] was made. None of the dietary changes or supplements of inorganic chemicals had any effect on her clinical condition. In spite of attempts at treatment the frequency of the tonic-clonic seizures increased over the years and the patient’s neurological condition deteriorated. Neither the incidence of the xanthine calculi nor the xanthine-to-hypoxanthine ratio changed after allopurinol medication.

The effect of dietary changes on the metabolism of sulfur amino acids was evident. Restriction of methionine and cysteine intake led to a decreased urinary output of abnormal sulfur metabolites, whereas the opposite was observed after loading with L-cysteine. This loading test did not provoke adverse clinical reactions like those reported by Irreverre et al. (22) in their patient with isolated sulfite oxidase deficiency.

Analysis of Hepatic Molybdenum and Molybdoenzymes.

As shown in Table 2, hepatic levels of sulfite oxidase and xanthine dehydrogenase activities in the patient were below the limits of detection of the assay procedures employed. In addition, atomic absorption analysis failed to detect any molybdenum in the liver sample. By neutron activation analysis the serum molybdenum concentration was found to be normal. The nonmolybdenum enzymes superoxide dismutase, glutamate dehydrogenase, monoamine oxidase, and alcohol dehydrogenase were found to be within normal limits.

Various procedures were employed to test for the presence of inactive sulfite oxidase and xanthine dehydrogenase proteins. The possible presence of nonfunctional sulfite oxidase protein capable of being activated by molybdate or by molybdenum cofactor was assayed as follows: 0.1 g of liver was homogenized in 0.5 ml of 10 mM potassium phosphate buffer, pH 7.4. An aliquot of the homogenate was diluted 1:10 in the same buffer and incubated with 2 mM sodium molybdate at 37°C for 45 min. A second aliquot was diluted 1:4 in the same buffer and mixed with an equal volume of an extract of Escherichia coli (20). The mixture was incubated at 37°C for 45 min. Active sulfite oxidase could not be detected in either incubation mixture.

Radioimmunoassay of sulfite oxidase crossreacting material (Table 2) indicated the presence of not more than 0.7% of the level found in a liver sample from a control individual of approximately the same age. In the control liver sample, the amount of crossreacting material detected by radioimmunoassay was in excellent agreement with the amount of active sulfite oxidase present. However, because the low value obtained by radioimmunoassay of sulfite oxidase in the patient could represent either an extremely depleted level of normal protein or relatively high levels of an altered product with low reactivity in this particular assay, two other immunologic procedures were applied.

The sulfite oxidase molecule exists as a dimer of identical subunits. Each subunit contains one cytochrome bs-type heme and one molybdenum cofactor moiety, but the two prosthetic groups are present on separate domains of the polypeptide chain (23). The heme-binding domain contains those antigenic sites that bind antibodies inhibitory to the catalytic activity (unpublished observations). Thus, an intact bs domain, preincubated with antiserum directed against sulfite oxidase, can deplete the serum of all inhibiting antibodies and protect native enzyme from inhibition during a subsequent incubation. By using this technique, it is possible to assay for the presence of an intact heme-binding portion of the sulfite oxidase molecule (13). The procedure was applied, using experimental conditions as described (13); no protection was observed, indicating the absence of the heme domain antigen in the tissue sample.

Sulfite oxidase crossreacting material was also sought by Ouchterlony double immunodiffusion. As shown in Fig. 1A, this technique also failed to detect any sulfite oxidase protein in the liver sample.

Xanthine dehydrogenase apoprotein was readily observable on immunodiffusion plates (Fig. 1B). Precipitin bands formed as lines of identity with active xanthine dehydrogenase present in liver tissue from control individuals and appeared to be equal in intensity. Bands were easily observable without staining but were intensified for photographic purposes by using an activity stain that requires functional flavin and Fe/S centers but is independent of the molybdenum cofactor (24). The xanthine dehydrogenase protein present in the liver sample could not be activated by incubation with molybdate or a source of molybdenum cofactor. Demolybdoxanthine dehydrogenase syn-

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**Fig. 1.** Ouchterlony double immunodiffusion of liver extracts versus antisera directed against sulfite oxidase (A) and xanthine dehydrogenase (B). Samples were prepared for immunodiffusion as follows: 0.1 g of liver was homogenized in 0.5 ml of 10 mM potassium phosphate, pH 7.8, and centrifuged at 35,000 × g for 15 min. The supernatant fraction was lyophilized and resuspended in 30 μl of H₂O. Each well contained 10 μl of extract. Wells numbered 4 contained extracts from control liver; wells numbered 5 contained extracts of the liver from the patient. Wells 1, 2, and 3 contained purified human sulfite oxidase (A) or partially purified human xanthine dehydrogenase (B). Antisera were in the center wells. The plates were allowed to develop for 2 days at room temperature, soaked in 0.9% NaCl for 2 hr, and stained for protein with amido black (A) or for activity by using 0.25 mg of iodonitrotetrazolium violet and 0.2 mg of NADH per ml of 50 mM potassium phosphate, pH 7.8 (B).
Table 3. Levels of sulfite oxidase activity and crossreacting material in cultured fibroblasts

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sulfite oxidase activity, units/mg</th>
<th>Sulfite oxidase crossreacting material, units/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n = 8)</td>
<td>0.0057 ± 0.0230 (average 0.0160)</td>
<td>-</td>
</tr>
<tr>
<td>Patient</td>
<td>&lt;0.0008</td>
<td>&lt;0.0008</td>
</tr>
<tr>
<td>Father</td>
<td>0.0094</td>
<td>0.0100</td>
</tr>
<tr>
<td>Mother</td>
<td>0.0089</td>
<td>0.0091</td>
</tr>
<tr>
<td>Brother</td>
<td>0.0088</td>
<td>0.0088</td>
</tr>
</tbody>
</table>

* Based on a specific activity of 918 units per mg of human sulfite oxidase.

Assays of Molybdenum Cofactor. In addition to the molybdenum cofactor present in the various molybdoenzymes, animal tissues normally contain a storage pool of the cofactor on the mitochondrial outer membrane (19). This pool of cofactor can be assayed by its ability to reconstitute sulfite oxidase activity in preparations of demolybdoenzyme isolated from livers of tungsten-treated rats (19) or by its ability to restore nitrate reductase activity to extracts of the Neisseria mutant nit-1 (unpublished observations). As shown in Table 2, both of these assay procedures failed to detect active cofactor in the liver sample from the patient. Because the liver sample was devoid of molybdenum, 10 mM sodium molybdate was included in the reconstitution mixtures.

Sulfite Oxidase Activity and Crossreacting Material in Cultured Fibroblasts. Results of activity measurements and radioimmunoassays of sulfite oxidase in fibroblasts cultured from the patient, parents, and a normal older brother are summarized in Table 3. Whereas both sulfite oxidase activity and crossreacting material were below limits of detection in the fibroblasts from the patient, the levels found in other members of the family were indistinguishable from normal.

Aldehyde Oxidase. Aldehyde oxidase is a molybdoenzyme that resembles xanthine dehydrogenase in physical properties and overlaps the latter enzyme in substrate specificities (25). In addition, available data strongly suggest that this enzyme also utilizes the molybdenum cofactor common to sulfite oxidase and xanthine dehydrogenase (1). However, the levels of aldehyde oxidase in human liver are extremely low (26), and the ability of the enzyme in frozen samples has precluded direct assay of this enzyme in the liver sample from this patient. Aldehyde oxidase activity was tested in vivo with a L-histidine loading test (100 mg/kg of body weight). Healthy controls metabolize a small fraction of the administered dose to hydantoin-5-propionic acid (27). Oxidation of the intermediate imidazolonepropionic acid to hydantoin propionic acid is catalyzed by an enzyme tentatively identified as aldehyde oxidase (28). The patient did not produce a measurable amount of hydantoin propionic acid after L-histidine loading, which may be considered indirect evidence of aldehyde oxidase deficiency.

**DISCUSSION**

The patient described above is the fourth individual identified with a deficiency in the ability to synthesize active sulfite oxidase. Like the other three (21, 22), this patient exhibits symptoms of mental retardation and neurological abnormalities and has dislocated ocular lenses. Results obtained from this patient concerning urinary and plasma levels of the various sulfur-containing metabolites were also in agreement with patterns observed previously and emphasize the usefulness of these characteristics in diagnosis of sulfite oxidase deficiency. Because xanthinuria in humans is known to be a relatively benign condition (29), the presence of urinary xanthine stones is probably the only clinical manifestation of xanthine dehydrogenase deficiency in this patient.

The finding of a combined deficiency of at least two molybdoenzymes in this individual must be considered a very rare observation. Although a similar biochemical situation has been produced experimentally in the rat by tungsten administration (30) and has been observed in mutant fungal systems (5, 6), an inborn error of molybdenum metabolism leading to the simultaneous deficiency of two enzymes in a human is most unusual. However, other biochemical systems are known in which one defect leads to the expression of two apparently nonrelated inborn errors of metabolism. For example, defects in cobalamin synthesis have been shown to result in the simultaneous occurrence of methylmalonic acidemia and homocystinuria (31, 32).

The biochemical data obtained thus far have indicated that this patient is unable to synthesize active molybdenum cofactor. It would appear likely that this results from a primary defect in an essential step in cofactor biosynthesis, although the exact nature of the defect remains to be established. Recent studies from this laboratory (2) have shown that the molybdenum cofactor contains a pterin nucleus with an as yet unidentified 6-alkyl side chain. In addition, it is known that the pteridine of the active cofactor is in a reduced state (2). It is not yet known whether animals can synthesize the cofactor de novo or whether all or part of it is a dietary requirement. Nonetheless, these findings suggest that a biochemically complex synthetic pathway would be required to produce active cofactor and that defects at any of several steps could lead to aberrant cofactor synthesis and the resulting combined deficiency disease. Indeed, if the biosynthesis of the pterin ring of the molybdenum cofactor occurs by the same pathway as that of tetrahydrobiopterin, the phenylalanine hydroxylase cofactor (33), one might envision a more complex combined deficiency disease that includes phenylketonuria. It would thus be of considerable importance to examine atypical phenylketonuric patients (34) for deficiencies of sulfite oxidase and xanthine dehydrogenase.

The apparent absence of sulfite oxidase apoprotein in both liver and cultured fibroblasts from this patient is an interesting finding that requires further analysis. Several possible explanations for this observation come to mind. The possibility that the primary defect in fact lies in the protein and leads secondarily to absence of cofactor is unlikely because at least two of the other sulfite oxidase-deficient patients appear to lack the apoprotein for the enzyme yet synthesize normal levels of active molybdenum cofactor (13, 21). A more likely alternative is that the molybdenum cofactor is required to specifically induce the synthesis of sulfite oxidase. However, the presence of apparently normal levels of xanthine dehydrogenase apoprotein in the liver sample would imply that the cofactor does not function as a positive regulator for this enzyme. A third possible explanation for absence of sulfite oxidase protein is that a deletion of genetic material required to direct synthesis of the molybdenum cofactor may have simultaneously eliminated elements necessary for sulfite oxidase biosynthesis. And finally, it is entirely possible

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4 During the preparation of this manuscript, two additional patients with combined deficiencies of sulfite oxidase and xanthine dehydrogenase have come to our attention. One has been identified in Paris by J.-M. Saudubray and one in London by S. Krywawych and F. Murray.
that molybdenum cofactor on the mitochondrial outer membrane is essential for internalization or "packaging" of the nascent sulfite oxidase in the mitochondrial intermembrane space. In the absence of this internalizing force, cofactorless sulfite oxidase in the cytosol may be perceived as a foreign protein and be rapidly degraded. Or, cytosolic enzyme lacking molybdenum cofactor may in addition be devoid of heme and in this bare state be totally unrecognized by antiserum directed against the holoenzyme.

The total absence of hepatic molybdenum in this individual raises the possibility that molybdate as such may not be present in human liver. If all molybdenum is normally present in cofactor form, either bound to molybdoenzymes or as the mitochondrial storage pool, then absence of cofactor could result in severe depletion of hepatic stores of the metal as observed in this patient. Such a situation does in fact obtain in the rat, in which the total hepatic content of molybdenum can be accounted for as molybdenum cofactor incorporated into sulfite oxidase and xanthine dehydrogenase and bound to the mitochondrial outer membrane (25). A final point to be considered, however, is the possibility that the absence of active molybdenum cofactor and sulfite oxidase protein are both in fact ramifications of molybdenum deficiency. Normal levels of serum molybdenum would rule against a dietary deficiency or an aberrant intestinal uptake system. Although we cannot eliminate the possibility of a specific defect in uptake of the metal at the level of the liver cell (and fibroblast), it is more likely that in the absence of functional cofactor to bind the metal, any molybdenum taken up by the cells is rapidly exported.

The data available presently do not establish whether the combined deficiency in this patient is in fact a heritable disorder. Because the molybdenum cofactor is normally synthesized in excess of requirements for known molybdoenzymes (13), synthesis of decreased amounts of active cofactor by heterozygous carriers of the defective gene may not be reflected as decreases in sulfite oxidase activity or protein. Only a direct assay of the storage pool of cofactor would reveal a difference. Unfortunately, sufficiently sensitive assays for cofactor levels in fibroblasts are not currently available.

It is clear that further studies on the structure of the molybdenum cofactor and the reactions involved in its synthesis will be required for a fuller understanding of the defect in the patient described above. In addition, studies of other patients with combined deficiencies of molybdoenzymes would be of extreme value in attempts to unravel the complex interactions between the molybdenum cofactor and the various molybdoenzymes in which it serves.

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