

Consequences of UMP synthase deficiency in cattle

(orotic acid/lactation/orotate phosphoribosyltransferase/orotidine 5'-monophosphate decarboxylase/perinatal mortality)

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ABSTRACT Several dairy cows have been identified as partially deficient in UMP synthase. Although erythrocytes of normal cows contained 2.54 units of enzyme per ml, four cows were discovered with only 1.08 units per ml. Cows deficient in UMP synthase secreted milk with abnormally high levels of orotate, 300–1,000 μg of orotate per ml compared to 80 $\mu\text{g}/\text{ml}$ for normal cows. The deficiency also was accompanied by a lactation-induced orotic aciduria. Although bovine urinary orotate was generally $<10 \mu\text{g}/\text{ml}$, the urine of the deficient cows, when lactating, contained 20–200 $\mu\text{g}/\text{ml}$. Their plasma orotate also was elevated. Genetic transmission of the condition was suggested by a common bull in the pedigrees of all deficient animals. Indeed, these cows, with half the normal level of UMP synthase, are probably heterozygotes with a 50% chance of passing the deficient allele to their progeny. For these putative heterozygotes, the condition is apparently benign because longevity and production were unaffected. However, the existence of a gene for UMP synthase deficiency in the dairy cow population poses a hazard with respect to the conception of homozygotic, deficient animals. These, in analogy with a comparable human condition, would be expected to exhibit high perinatal morbidity and mortality.

In mammalian cells, the last step of pyrimidine nucleotide synthesis involves the conversion of orotate to UMP and is catalyzed by UMP synthase (1). This multifunctional enzyme is comprised of two sequential activities, orotate phosphoribosyltransferase [orotate + phosphoribosylpyrophosphate \rightarrow orotidine 5'-monophosphate (OMP)] and OMP decarboxylase (OMP \rightarrow UMP + CO_2). A severe deficiency of this enzyme results in hereditary orotic aciduria, a rare disorder in humans. In addition to urinary excretion of massive quantities of orotate (1,500 mg/day vs. 1.4 mg/day in normal subjects), it is characterized by megaloblastic anemia and growth retardation (2). The latter conditions result from de facto starvation for pyrimidines and hence inadequate nucleic acid metabolism. Unless treated with exogenously supplied pyrimidines (usually uridine), afflicted individuals die in the neonatal period.

Orotate is a normal constituent of bovine milk and is virtually the only pyrimidine or purine in the acid-soluble fraction (3). Orotate concentrations in milk exhibit considerable variability; although some variation is due to breed, stage of lactation, and number of lactations, it is attributable in large measure to individual variation (4). In a multibreed dairy herd with 250 milking cows monitored for 5 yr, several cows were identified with persistently high levels of milk orotate. The herd mean (\pm SEM) was $81.1 \pm 15.8 \mu\text{g}$ of orotate per ml, yet milk from certain cows had $>300 \mu\text{g}/\text{ml}$ and, in one case, it reached nearly $1,000 \mu\text{g}/\text{ml}$.

The purpose of this study was to assess an enzymatic basis for the elevated secretion of orotate by these cows. It was hypothesized that a deficiency of UMP synthase would lead to

excess accumulation of orotate in milk. The erythrocyte enzyme was examined as it is readily available and is considered to be an indicator of relative enzyme levels in various body tissues, including the mammary gland. The latter organ has been identified as the source of milk orotate on the basis of whole animal (5), cell culture (6), and tissue slice (7) experiments. Upon identifying bovine UMP synthase deficiency, its consequences for the affected animal and for the dairy cattle population were explored.

MATERIALS AND METHODS

Blood samples were collected in heparinized tubes from the tail veins of selected cows and were placed on ice. After centrifugation at $650 \times g$ at 4°C and aspiration to remove plasma and leukocytes, the erythrocytes were washed twice with 0.9% NaCl. Hemolysates were prepared by mixing the washed cells with an equal volume of glass-distilled water. After centrifugation to remove cell debris, the hemolysates were stored in 0.5-ml aliquots at -22°C for subsequent analysis. UMP synthase stored in this manner was stable for at least 1 yr. Immediately prior to assay, the hemolysates were diluted with 2 mM Tris-HCl, pH 7.4/2 mM dithiothreitol and were held on ice for 30 min to ensure full activity. The assay used was that of Reyes (8), modified by inclusion of dithiothreitol. Final concentrations of components were: 50 mM Tris-HCl (pH 7.4), 5 mM MgCl_2 , 1 mM phosphoribosylpyrophosphate, 2 mM dithiothreitol, and 0.10 mM $[6\text{-}^{14}\text{C}]\text{orotate}$ (46.9 Ci/ μmol ; 1 Ci = 3.7×10^{10} Bq) in a total volume of 110 μl (37°C). The conversion of radioactivity from orotate to OMP and UMP was determined by chromatography on PEI-cellulose, elution, and quantitation in a scintillation counter. The assays were conducted in duplicate on separate days for each sample and were corrected for zero-time controls; UMP production was linear at 15 and 30 min for each sample. Samples collected a month or more apart had comparable activity. Values were averaged for all collections from each animal.

A representative milk sample was collected at the time of regular machine milking and was frozen until analyzed. Urine samples were collected on the same day as milk, filtered through membrane filters (0.45- μm pore size), and frozen. For quantitative urine collections, urinary catheters were inserted and urine was drained into large plastic containers with 100 ml of 8 M H_2SO_4 added as preservative. Urine was measured twice daily and aliquots were frozen for later analysis. Orotate was routinely assayed in deproteinized milk and filtered urine by the colorimetric method of Adachi *et al.* (9). Samples with elevated orotate also were analyzed by HPLC; in no case was orotidine detected at 1% of the level of orotate.

The cattle studied were of the Holstein-Friesian breed from the University of Illinois dairy herd. Animals were chosen for study based on milk orotate concentration and pedigree infor-

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Abbreviation: OMP, orotidine 5'-monophosphate.

Table 1. Erythrocyte UMP synthase, milk orotate, and urinary orotate in Holstein dairy cows

Cows*	UMP synthase, $\mu\text{mol/hr/ml}^\dagger$	Milk orotate, $\mu\text{g/ml}^\ddagger$	Urinary orotate, $\mu\text{g/ml}^\ddagger$
Normal			
2994	2.34	98.1	9.4
3445	2.16	85.9	—
3553	2.93	251.4	8.6
3660	2.42	58.7	6.1
3771	2.35	86.4	2.9
3872	2.98	85.9	6.7
3929	2.42	70.1	7.0
4050	3.04	200.4	10.3
4061	2.32	100.4	10.2
4191	2.45	173.5	9.6
Mean \pm SEM	2.54 \pm 0.10	121.1 \pm 20.3	7.9 \pm 0.8
Deficient			
2778	0.73	958	207.0
3365	1.29	346	24.4
3768	1.21	496	34.7
4043	1.10	718	84.8
Mean \pm SEM	1.08 \pm 0.12	630 \pm 134	87.7 \pm 41.9

* Individual cows are identified by number.

† UMP synthesized, $\mu\text{mol/hr/ml}$ of packed erythrocytes.

‡ Values represent peak concentrations.

mation; some normal cows were paired with deficient ones on the basis of age, calving date, number of lactations, and milk production. Usual feeding and management practices were followed; the same regimen was applied to animals being compared in longitudinal and total collection studies.

RESULTS AND DISCUSSION

Activities of erythrocyte UMP synthase in 14 dairy cows are summarized in Table 1. Ten cows, designated normal, had an average activity of 2.54 units/ml, whereas four others were partially deficient in UMP synthase. They averaged 1.08 units/ml or slightly less than half the normal level. As no differences in erythrocyte protein content were noted among the cows, the

deficiency is equally evident when activity is expressed per milligram of protein. Our control value (10.7 units/mg of protein) is consistent with that of Tax *et al.* (10), who reported 11.6 units/mg of protein for orotate phosphoribosyltransferase in bovine erythrocytes.

Also shown in Table 1 are peak concentrations of orotate in the milk of these cows. The normal cows peaked at 121 $\mu\text{g/ml}$, which was consistent with the herd mean of 81.1 $\mu\text{g/ml}$ (4). All deficient cows peaked at over 340 $\mu\text{g/ml}$ (mean = 630), linking UMP synthase deficiency to high levels of milk orotate in the bovine. However, despite normal UMP synthase, one cow (3553) had a milk orotate level as high as 250 $\mu\text{g/ml}$, demonstrating that factors other than UMP synthase level can be involved in elevated secretion of orotate in milk. The amount of this enzyme also cannot explain the species specificity of orotate secretion into milk. Although nonruminant animals generally have little or no orotate in their milk (11), UMP synthase activity per milligram of mammary tissue protein is higher, rather than lower, in cows compared to rats (12). Humans who consume milk with abnormally high concentrations of orotate (such as that from the deficient cows) are not likely to be at risk, in view of the observed species specificity, for consequences of orotate ingestion (13). Dietary orotate leads to severe fatty livers in the rat but not in any other species examined.

Finally, Table 1 lists urinary orotate measured in these animals. Normal animals had consistently $<10 \mu\text{g/ml}$, whereas the deficient cows peaked at 24–207 $\mu\text{g/ml}$ of urine. Thus, orotic aciduria is associated with UMP synthase deficiency in cows as it is in humans. However, this symptom was evident only when the cows were lactating (Fig. 1). Cows 2778 and 2994 were both in their eighth lactation, were consuming the same diet, and were producing similar levels of milk. Cow 2778 was deficient in UMP synthase and secreted the most milk orotate of any cow in the herd, whereas cow 2994 was normal for UMP synthase and milk orotate. As can be seen in Fig. 1, urinary orotate was constant (7.1 $\mu\text{g/ml}$) from 10 wk prepartum to 27 wk postpartum for cow 2994. Cow 2778 excreted urine that was indistinguishable in orotate concentration in the prepartum period. However, urinary orotate increased significantly and averaged 67.2 $\mu\text{g/ml}$ during the first 27 wk postpartum. A 6-day total collection of milk and urine conducted at week 17 revealed that

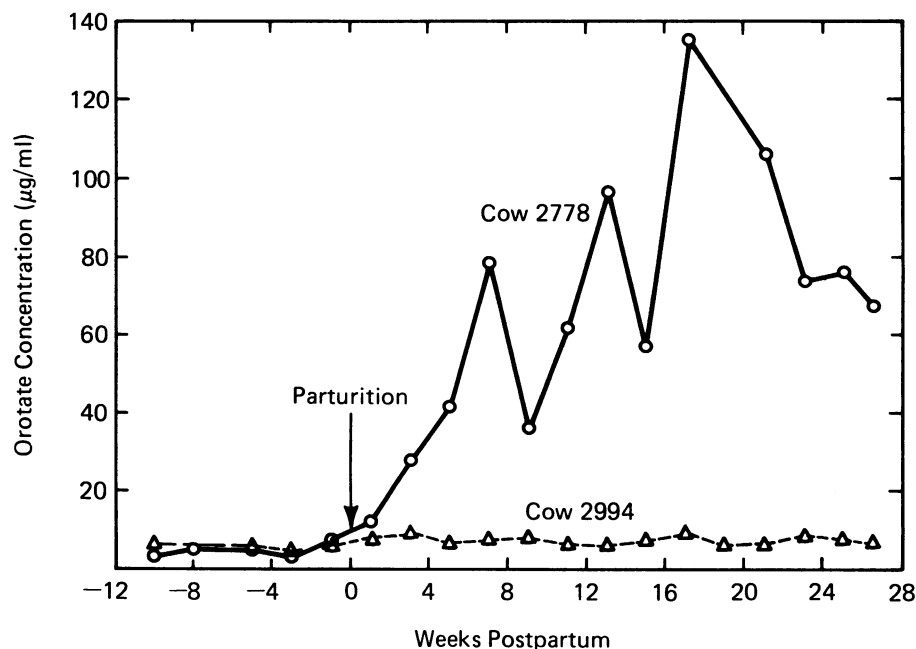


FIG. 1. Time course for excretion of urinary orotate in a normal cow (2994) and in one deficient for UMP synthase (2778).

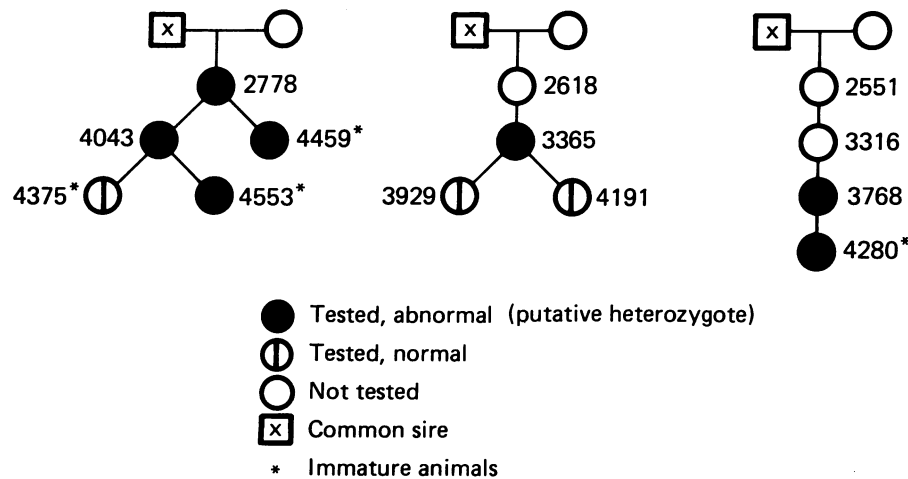


FIG. 2. Pedigrees of three families with UMP synthase deficiency. Bulls other than the common sire and untested progeny not in the direct line of descent are not indicated. Individual cows are identified by number.

cow 2778 secreted daily 19.4 g of orotate in milk and 2.3 g in urine, whereas cow 2994 released only 2.2 g in milk and 0.2 g in urine. At this same time, blood orotate was 586 ng/ml of plasma for cow 2778 compared to 67 ng/ml for cow 2994. Thus, UMP synthase deficiency leads to elevated orotate in milk, urine, and blood in this cow. Similar data were obtained in another pair of animals monitored over a complete lactation.

Because cow 2778 did not conceive in the first 27 wk of this lactation, the orotic aciduria is clearly induced by lactation rather than pregnancy. A mild orotic aciduria occurs during pregnancy in humans and rats (14–16), but no such phenomenon was evident in normal or UMP synthase-deficient cows. It is of interest that a moderate UMP synthase deficiency results in a lactation-induced orotic aciduria. Lactation requires enhanced utilization of pyrimidine nucleotides for synthesis of milk lactose (from UDP-galactose) and milk protein (via RNA); it can be hypothesized that, in the mammary gland with deficient UMP synthase, the extra demand for pyrimidines results in increased orotate secretion into milk. Urinary orotate might be similarly derived from the mammary gland; more likely it is made in the liver, where increased metabolism accompanying lactation may lead to orotate accumulation and its excretion in urine.

In addition to the four mature cows, three of their offspring have also been identified as deficient in UMP synthase. Because the young animals are not lactating, it is not yet known if they will secrete elevated orotate in milk or urine. The pedigrees of all animals identified as deficient are summarized in Fig. 2. Six daughter–dam pairs and a common sire for each pedigree are shown and imply genetic transmission of the condition. Prepotency was not shown because several daughters (3929, 4191, 4375) did not share the phenotype. Indeed, the deficient animals, with half the normal level of UMP synthase, were probably heterozygotes with a 50% chance of passing the deficient allele to their offspring. In the human deficiency, heterozygotes have half the normal enzyme activity in their erythrocytes (2).

For the apparently heterozygotic cows, the condition appeared benign. Numerous production characteristics for these cows were normal. Longevity was not compromised as cow 2778 at 13 yr of age is the oldest cow in the herd. However, the existence of a gene for deficient UMP synthase in the dairy cow population poses a hazard with respect to the conception of homozygotic, deficient animals. This, in analogy to the human condition, would be highly deleterious. Gene frequency of the deficiency in Holstein cattle is unknown. Even if the frequency is low, the wide use of artificial insemination in the dairy industry carries the potential for rapid and covert spread of the

gene. In a herd with no carrier cows inseminated with heterozygous bulls for two generations, no overt expression would be noted in the first generation, whereas, in the second, loss of 12.5% of the calves to perinatal morbidity and death would be expected. This would have adverse effects on the dairy farmer and, eventually, the consumers of dairy products. Furthermore, the gene frequency may not be as small (<1%) as it appears in the herd studied, raising the possibility that UMP synthase deficiency may already be responsible for unexplained cases of herd-specific high calf mortality (17). In addition, the errors of metabolism due to the homozygous condition could contribute to postconception embryonic and fetal deaths that lead to decreased fertility (18).

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- McClard, R. W., Black, M. J., Livingstone, L. R. & Jones, M. E. (1980) *Biochemistry* **19**, 4699–4706.
- Smith, L. H., Jr., Huguley, C. M., Jr., & Bain, J. A. (1972) in *The Metabolic Basis of Inherited Disease*, eds. Stanbury, J. B., Wyngaarden, J. B. & Fredrickson, D. S. (McGraw-Hill, New York), 3rd Ed., pp. 1003–1029.
- Johke, T. (1978) in *Lactation: A Comprehensive Treatise*, ed. Larson, B. L. (Academic, New York), Vol. 4, pp. 513–522.
- Jesse, B. W., Anderson, C. R. & Robinson, J. L. (1980) *J. Dairy Sci.* **63**, 235–242.
- Ahmed, A. A., Porter, G. A. & McCarthy, R. D. (1978) *J. Dairy Sci.* **61**, 39–43.
- Larson, B. L. (1976) *J. Dairy Sci.* **59**, 1881–1889.
- Ehle, F. R., Robinson, J. L., Clark, J. H. & Baumrucker, C. R. (1981) *J. Dairy Sci.* **64**, 2192–2196.
- Reyes, P. (1977) *Anal. Biochem.* **77**, 362–369.
- Adachi, T., Tanimura, A. & Asahina, M. (1963) *J. Vitaminol.* **9**, 217–226.
- Tax, W. J. M., Veerkamp, J. H. & Trijbels, J. M. F. (1976) *Comp. Biochem. Physiol. B* **54**, 209–212.
- Larson, B. L. & Hegarty, H. M. (1979) *J. Dairy Sci.* **62**, 1641–1644.
- Chen, M.-H. & Larson, B. L. (1971) *J. Dairy Sci.* **54**, 842–846.
- Durschlag, R. P. & Robinson, J. L. (1980) *J. Nutr.* **110**, 822–828.
- Wood, M. H. & O'Sullivan, W. J. (1973) *Am. J. Obstet. Gynecol.* **116**, 57–61.
- Milner, J. A. & Visek, W. J. (1978) *J. Nutr.* **108**, 1281–1288.
- Harris, M. L. & Oberholzer, V. G. (1980) *Clin. Chem.* **26**, 473–479.
- Otterby, D. E. & Linn, J. G. (1981) *J. Dairy Sci.* **64**, 1365–1377.
- Salisbury, G. W., Hart, R. G. & Lodge, J. R. (1977) *Am. J. Obstet. Gynecol.* **128**, 342–350.