

U.S. Department of Agriculture Accomplishments Report AD-421 U.S. Dept. of Agriculture, State Agricultural Experiment Stations and Other Institutions			Date (Month, Day, Year) 02/18/2008
1. Accession 0201388	Agency Identification No. 2. CSREES 3. IOW	5. Work Unit/Project No. IOW05009	6. Status Annual Report
7. Title Heartland Integrated Water Quality Coordination Initiative			
12. Investigator Name(s) (Last Name and Initials) Miller, G. A.; Lawrence, J. D.; Wright Morton, L.			
20. Termination Date 09/15/2008		40. Period Covered (mo/da/year): 01/01/2007 TO 12/31/2007	
Outputs: <p>In September the Heartland leadership involved the Extension to Agriculture and Natural Resources leaders and the regional advisory committee in a meeting held at Region 7 EPA Headquarters to discuss and prioritize regional water issues. At that time the EPA Regional Administrator John B. Askew presented the Heartland participating universities with Regional Leadership Awards recognizing their productive efforts to address priority water quality issues and increase support from partners. An EPA-prepared press release was highlighted by the national CSREES water program. Heartland issue teams conducted seven roundtables and workshops, published an extension circular, two research papers, a Master's thesis, a book chapter, two reports in conference proceedings and four state technical reports on the Regional Water Survey. Results of the survey were the subject of the regional poster and a workshop presentation at the CSREES National Water Conference, to which Heartland teams also contributed six other presentations and two posters. The AMM issue team conducted an ethanol byproducts roundtable with scientists and ethanol producers discussing expected implications of feeding distillers grains on nutrient management plans. Six fact sheets are under development as a result. The team also published four issues of the on-line AMM Newsletter, created resources for a regionally-adapted model Nutrient Management Plan and virtual tours of Alternative Technologies. The NMP plan has been used for training and in discussions of proposed changes to national and state AFO rules. "Living with your permit" workshops were held in conjunction with ISU Extension to assist NPDES permit holders and those in the process of receiving a permit. Consultants and agency staff were also among the 120 attendees. The annual AMM conference included updates on the revised CAFO Rule Proposal, CNMP/NMP issues, feeding of ethanol co-products and alternative technologies. A roundtable on Environmental Performance Measures for AFO Nutrient Plans initiated an attempt to develop regional recommendations for the best available indicators of success. The NPM team conducted a regional research roundtable and a workshop on Targeting Critical Areas for Implementation of BMPs. The objective was to review current research, compile, evaluate and promote increased understanding of BMP targeting and assessment processes and information. Topics included remote sensing and GIS, modeling, economics, conservation effects, the human dimension and agency perspectives. The workshop included field tours on land and stream assessment. The team also collaborated with the CIWM issue team and a 406 integrated project, "Pollution and Economic Decision Support Tool" to conduct a workshop "Stakeholder Involvement in Watershed Management in Eastern Nebraska" for technical professionals and extension educators to learn approaches and methods for involvement of communities. CIWM research by ISU included key informant surveys in 75 Missouri and Iowa watersheds. Presentations and materials for all Heartland workshops are available from the Initiative home page www.heartlandwq.iastate.edu.</p>			
Outcomes/Impacts: <p>Heartland issue teams have impacted technical, educational and regulatory programs. In addition to dissemination of research-based information, Heartland has helped university extension become widely recognized for capacity building as convenor and facilitator of partnerships and ongoing dialog among agencies, universities and other organizations on water resource topics of regional concern. Collaboration by Heartland teams has also resulted in increased integration of the human dimension into the work of other technical groups. In 2007 both the AMM and NPM teams saw increased interest and participation of partner organizations in their regional workshops. On a feedback questionnaire at the AMM Annual Conference, over 90 percent of respondents said that at least five of the eight focus topics provided information useful for their work and provided a perspective that helped them frame the issues. Topics of greatest interest (100%) were</p>			

performance measurement, ethanol issues and vegetative treatment systems. They expected to take action: to share information with colleagues (77%), communicate with increased confidence with the AFO industry (65%), share information with new contacts in other organizations (50%); incorporate regional resources into their programs (44%); and engage policy makers in their state (43%). AMM has also had a direct impact on the interaction of regulatory programs and the livestock industry. The regional response to EPA's 2006 CAFO Proposal has influenced CNMP management options presented in the 2007 Proposed CAFO Rule. The outcome will be a policy more consistent with land grant university recommendation and more likely to be implemented by producers. Collaborative research and demonstration involving the AMM team has resulted in EPA Region 7 acceptance of states' permitting Vegetative Treatment Systems, on a case-by-case basis, for the first time in over 30 years of CAFO regulation. On the feedback questionnaire at the NPM Targeting workshop participants rated the event very highly on improving their awareness. New technologies and approaches to targeting were overall the items of highest interest, while the "Human Dimension" presentation received the highest score for "importance in my line of work". Over 75 open-ended comments were recorded. Following the Targeting workshop and field tours, the EPA 319 Program coordinator decided to provide computer resources and training for improved stream assessment to all Heartland states. This investment leveraged by Heartland will improve watershed management planning throughout the region. Lois Wright Morton, ISU Department of Sociology, provided principal leadership for publication of the 2007 Regional Water Issues Survey technical reports. The IDNR has used the results to inform policy makers about the awareness and needs of lowans for more responsive environmental programs. The survey also influenced ISU Extension program teams in their development of the 2008-2012 Plan of Work. The new Plan places greater emphasis on natural resources and environmental stewardship education, including water quality, and addresses emerging priority issues and non-traditional audiences.

Publications:

Corey, K. 2007. The endangered species act, local power and contested issues on the rural-urban interface. M.S. Thesis, Iowa State Univ., Ames.

Morton, L.W. 2007. Getting to better water quality outcomes: The promise and challenge of the citizen effect. Lyson Memorial Volume, Cornell University (In press).

Morton, L.W. and Brown, S. 2007. Water issues in Iowa: A survey of public perceptions and attitudes about water. The Heartland Regional Water Coordination Initiative Bulletin #SP290 Iowa State University Extension.

Morton, L.W. and Brown, S. 2007. Water issues in Nebraska: A Survey of public perceptions and attitudes about water. The Heartland Regional Water Coordination Initiative Bulletin #SP291 Iowa State University Extension.

Morton, L.W. and Brown, S. 2007. Water issues in Kansas: A survey of public perceptions and attitudes about water. The Heartland Regional Water Coordination Initiative Bulletin #SP292 Iowa State University Extension.

Morton, L.W. and Brown, S. 2007. Water issues in Missouri: A survey of public perceptions and attitudes about water. The Heartland Regional Water Coordination Initiative Bulletin #SP293 Iowa State University Extension.

Wortmann, C.S. 2007. Soil and water summaries for 2005 to 2006. NebGuide G1719, University of Nebraska-Lincoln.

Wortmann, C.S. and D.A. Walters. 2007. Residual effects of compost and plowing on phosphorus and sediment in runoff. J. Envir. Qual. 36:1521-1527.

Mamo, M., Wortmann, C., and Brubaker, S. 2007. Manure P Fractions: Analytical methods and the effect of manure types. Comm. Soil and Plant Anal. 38:935-947.

R. Koelsch, Massey, R., Erickson, G., and Bremer, V. 2007. Software Tool for Integrating Feed Management. Proceedings of the International Symposium on Air Quality and Waste Management for Agriculture. Broomfield, CO.

Massey, R., Applegate, T., and Koelsch, R. 2007. Economics Of Land Application Of Laying Hen Manure. [unpaginated CD-ROM]. Proceedings of the Multi-state Poultry Feeding and Nutrition Conference. Indianapolis, IN.

Participants:

The Heartland Initiative was developed by extension/research faculty, including the Extension Water Quality Coordinators, of the region's land grant institutions - Iowa State University, Kansas State University, the University of Missouri, and the University of Nebraska-Lincoln - departments of agronomy, agricultural engineering, economics, forestry and sociology. The Heartland leadership team also includes a representative of the U.S. EPA Region 7 Watershed Planning and Implementation Branch. The overall goal of the Heartland Initiative is to build institutional partnerships and increase the capacity of citizens, landowners, agencies and community leaders to better address water quality concerns. Therefore, Heartland activities are implemented by regional "issue teams" made up of technical, regulatory and research specialists from state departments of agriculture and natural resources, NRCS, EPA R7 and Heartland universities. To date more than 200 people, half of whom are associated with land grant universities, have contributed their time and expertise to the planning, management and implementation of Heartland roundtables and workshops, and as authors of regional publications and newsletters. Successful multi-state water research and extension projects have grown from partnerships developed by each of the teams. A Regional Stakeholder Advisory Committee is made up of representatives of the Iowa Farm Bureau, the EPA Region 7 Ag Advisor, Director of the Nebraska Water Center, Kansas Department of Health and Environment and the Missouri Department of Natural Resources. The Advisory Committee provides advice and support on strengthening the Heartland Regional Initiative, shares perspectives on emerging water issues, helps build new partnerships and collaborations, and helps the Heartland Regional Leadership Team communicate its goals and progress.

Target Audiences:

Multiple audiences are targeted for capacity building by Heartland issue teams, including members of land grant research and extension programs, technical and regulatory agencies, non-governmental organizations, advisors to the crop and livestock industry, and citizen watershed leaders. Over 400 people have participated in one or more Heartland events. Some Heartland regional activities focus specifically on capacity building for university extension, including 1890 and 1994 universities, to take an expanded role in water quality and other natural resources outreach and education. Focus group studies document Heartland Initiative impacts on institutional capacity. Partners recognize Heartland as a facilitator of regional networks and partnerships as well as a source of research-based knowledge. Further, environmental agency staff and researchers report greater awareness of related work in other organizations and states, and are more likely to seek out land grant resources. Participants report that Heartland has given them increased access to EPA staff and programs.

Project Modifications:

None Reported

Approved (Signature)	Title	Date

U.S. Department of Agriculture Accomplishments Report AD-421 U.S. Dept. of Agriculture, State Agricultural Experiment Stations and Other Institutions			Date (Month, Day, Year) 02/18/2008
1. Accession 0201724	Agency Identification No. 2. CSREES 3. IOW	5. Work Unit/Project No. IOW05016	6. Status Annual Report
7. Title Educational Program to Increase Citizens' Responsibility for Management of Agricultural Watersheds			
12. Investigator Name(s) (Last Name and Initials) Miller, G. A.; Morton, L. W.			
20. Termination Date 09/14/2008		40. Period Covered (mo/da/year): 01/01/2007 TO 12/31/2007	
Outputs: <p>Councils in the Hewitt, Lime and Coldwater/Palmer Creek watersheds met monthly during 2007, with breaks for field work in spring and fall. They reviewed progress through cooperators' performance index results, monitoring and other information, approved incentives and set goals for their 2008 programs. There were 111 participants in the performance program during 2007. They conducted over 180 stalk nitrate tests and were assisted by the project computer specialist to calculate the Phosphorus Index and Soil Conditioning Index on 829 fields. Cooperators earned a total of \$131,818 in incentive funds. The project is also collaborating with Dr. Jonathan Winsten, Winrock International and University of Vermont, on an NRCS Conservation Innovation Grant (CIG) Pilot-testing Performance-Based Incentives for Agriculture Pollution Control, an economic and policy study to evaluate the cost effectiveness of payments for farm-level performance targets. The project coordinator facilitated formation of a watershed residents' council in the Coffee Creek watershed and conducted a series of meetings between Dr. Winsten and the council to initiate their program. Compared to this project, the CIG project places more emphasis on quantitative environmental outcomes and less on the ability of performance measures to promote social/educational outcomes - growers' voluntary adoption of practices to achieve continuous environmental improvement. The Coffee Creek council, with assistance from the project coordinator, also submitted a proposal to the Iowa Watershed Improvement Review Board to extend their project beyond the CIG support for one year and 13 cooperators to a watershed-wide, three year program. As a result of the project's contact with northwest Iowa Extension staff, first reported in 2006, a group went on to develop a successful proposal for a Watershed Development Planning and Assistance grant from the Iowa Department of Agriculture and Land Stewardship Division of Soil Conservation. The grant will support a specialist to develop a more comprehensive proposal for conservation work, including producer performance programs, in the Willow Creek watershed. Information about the project was shared at the CSREES National Water Conference and numerous other state and regional conferences and workshops. The watershed councils' efforts also resulted in extensive press coverage in local and national media. Press reports are available on the project website and at http://www.heartlandwq.iastate.edu/RegionHome/CSREESProjects/01watershdmgt/3602004Miller.htm.</p>			
Outcomes/Impacts: <p>An advantage of the locally-directed performance program is the ability of each council to set its own nonpoint source performance targets and incentives based on local conditions and each year's progress. This flexibility has been a major contributor to the success of the program, which grew by 46% in 2007 to a total of 111 participants including 45-55% of the growers in each of the three watersheds. Stalk nitrate tests conducted in 2007 showed average test scores significantly reduced from baseline values. In Hewitt Creek the average score was reduced 33% between years 1 and 2 and an additional 29% between years 2 and 3. All the watersheds provide a small incentive for manure nutrient analysis and spreader calibration. The result has been growers' increased confidence in use of manure credits, moving manure further from the livestock facilities, and interest in local manure bartering arrangements. The impact of performance feedback on individual practice choices has also been very cost effective for improved soil management. Cooperators have now installed or improved 22 miles of waterways or buffers on 19,200 acres of cropland, with significant impact on their PI and SCI scores, for a total incentive expenditure of only \$16,700. More headlands and farmable berms were added as soil loss control management incentives. One project has added an incentive for a 40' x 40' or larger grass buffer at the field edge point of concentrated flow to keep soil and nutrients from leaving the field. The buffers must be maintained at least 3 years. In Hewitt Creek watershed the spring and</p>			

fall macroinvertebrate evaluations continued to reflect slightly improved diversity and numbers of populations that can survive only in improved water conditions. Watershed councils have taken many opportunities to communicate their engagement and enthusiasm for the program to their elected representatives. In late December, the Hewitt Creek council submitted a request through Senator Charles Grassley for seven additional years funding for their watershed and the North Fork Maquoketa Headwaters. Their request includes support for staff to continue the group facilitation, education and administrative assistance currently provided by this project. While the performance incentives are an education-based program, the administrative support provided under this CSREES grant is also crucial to make it possible for volunteer councils to meet extensive reporting requirements of public programs like the Iowa WIRB.

Publications:

Morton, L.W. 2008. The Role of Civic Structure in Achieving Performance Based Watershed Management. Society & Natural Resources (In press). Morton, L.W. and Miller, L.W. 2007. Connecting Sustainable Agriculture to Rural Development: The Case of Pasture-Based Dairy Grazing. Journal of Community Development 38:3:23-38.

Morton, L.W. 2007. Lower Big Sioux River Watershed Survey. Iowa State University Extension, Department of Sociology Technical Report No. 1009; Individual subwatershed Technical Reports No.1010-1020.

Participants:

The project involves the efforts of voluntary citizens watershed councils in three northeast Iowa watersheds and over 100 farmers cooperating in their locally-directed performance-based environmental management incentive programs. Extension watershed specialists provide group facilitation and technical assistance as an educational program to the incentive cooperators. The funds used by the local councils for their performance incentives have been provided by the Iowa Corn Growers, the Iowa Farm Bureau and the Iowa Watershed Improvement Review Board (WIRB). The project coordinator is funded by the USDA CSREES National Integrated Water Quality Program. An extension nutrient management/computer specialist and an information specialist are funded by a U.S. EPA Region VII Watershed Improvement grant. Sociological surveys of program cooperators are being conducted by co-PI Dr. Lois Wright Morton, Iowa State University. Limited watershed monitoring is an in-kind contribution by local college environmental studies programs. Assistance to initiate a performance program in an additional watershed has been provided by an NRCS Conservation Innovation Grant. Details about the performance program and consultation with project staff have been made available through the project's participation in workshops sponsored by the Heartland Regional Water Coordination Initiative and at request to project staff and local watershed leaders. John Rodecap, ISUE Performance-based Watershed Projects Coordinator, is the principal contact (jrodecap@iastate.edu, 563-425-3233).

Target Audiences:

Farm operators in priority watersheds are concerned about being targeted by regulatory programs and want to act on their own environmental goals and objectives. Locally-directed performance-based management incentives provide them and their communities with a way to evaluate their actual environmental impact and progress resulting from improved management. In addition to growers and their neighbors who participate directly, other audiences for this work are leaders of commodity and farm-advocacy organizations, elected officials and environmental and conservation agency programs.

Project Modifications:

None Reported

Approved (Signature)	Title	Date

U.S. Department of Agriculture Accomplishments Report AD-421 U.S. Dept. of Agriculture, State Agricultural Experiment Stations and Other Institutions			Date (Month, Day, Year) 02/18/2008
1. Accession 0194795	Agency Identification No. 2. CSREES 3. IOW	5. Work Unit/Project No. IOW06625	6. Status Final Report
7. Title Enhancing the Competitiveness of U.S. Meats			
12. Investigator Name(s) (Last Name and Initials) Olson, D. G.; Hayes, D. J.; Sapp, S. G.			
20. Termination Date 09/30/2007		40. Period Covered (mo/da/year): 10/01/2002 TO 09/30/2007	
Outputs: <p>Oxidation of mu-calpain results in inactivation of calpain, little myofibrillar fragmentation and little improvement in tenderness and water holding capacity. The results demonstrate that pH decline and protein oxidation are sources of variation in postmortem proteolysis and associated development of meat water holding capacity and tenderness. It has been documented that lower pH (5.6, 6.0, 6.5, and 7.0) significantly alters gelation properties of myofibrillar protein in solution supporting a relationship between pH and the textural properties of porcine myofibrillar protein gels. Feeding Vitamin E to market animals may not only prevent premature off-color development in fresh beef and poultry, but may also provide some protection from oxidative conditions leading to improved tenderization of beef. It was demonstrated that pork proteins mixtures are be softened with addition of a modified pork collagen ingredient, thus improving the texture of low-fat products. These results document that increasing the utilization and value of a relatively low value pork meat component is feasible. Results suggest that high pH pork (above pH 5.8) can be expected to be superior in quality to lower pH pork. At high pH, greater lipid content does not improve quality. At low pH (below pH 5.5), pork is of inferior quality. At low pH, greater lipid content does not improve sensory quality. Lipid content within the intermediate pHs indicate that only a small portion of sensory quality is affected. The combination of CO and CO₂ in MAP was beneficial in extending the shelf life of pre-rigor and post-rigor fresh pork sausage by reducing aerobic and psychrotrophic microbial growth, and improving oxidative stability and color, compared to conventional OW packaging. Ham slices stored at 4 degrees C, treatment with 25 µg of the colicin ColE1 reduced Listeria growth below detection limits for the slices inoculated with 4 log CFU/ml for 14 days. ColE1 is highly effective against Listeria. A conjoint analysis method was used examine the relative utilities of a set of beef steak characteristics. Region of origin is by far the most important characteristic followed by animal breed, traceability, animal feed, and beef quality. Alternatively, the cost of cut, farm ownership, the use of growth promoters, and whether the product is guaranteed tender were the least important factors. The ideal steak for the national sample is from a locally produced, choice Angus fed a mixture of grain and grass that is traceable to the farm of origin. If the product was not produced locally, respondents indicated that their preferred production states are, in order from most to least preferred, Iowa, Texas, Nebraska, and Kansas.</p>			
Outcomes/Impacts: <p>Processors that adapt technologies that increase the ultimate pH of pork loins could expect to have improved sensory quality, texture, and cook loss. Pork loins that have higher lipid content have improved sensory traits at lower ultimate pH levels although the loins were inferior to higher pork loin pH. Therefore, pork processors should adapt technologies that increase pH. Processors adapting the use of CO in MAP packaging would find benefits in extending the shelf life of pre-rigor and post-rigor fresh pork sausage by reducing aerobic and psychrotrophic microbial growth, and improving oxidative stability and color, compared to conventional OW packaging. Processors that alter pH to change gel strength has specific applications in processed meats that can improve the texture of low-fat products. Documentation that oxidation and pH have specific control over development of fresh meat quality (specifically tenderness) is an important result that will lead to applications that reduce the variation in oxidation of meat and improvement in meat tenderness. Results have already allowed meat ingredient processors to develop new applications for pork collagen in processed meat systems to lower costs to their meat formulation. Colicins were shown to be effective in controlling growth of Listeria monocytogenes and, when approved for food products, would be a useful tool to control this organism. Consumer study has shown that those marketing beef could use the region of origin as a compliment to their marketing plan.</p>			

Publications:

Patton, B.S., J. S. Dickson, S. M. Lonergan, S. A. Cutler, and C. H. Stahl. 2007. Inhibitory activity of Colicin E1 against *Listeria monocytogenes*. *J. Food Protect.* 70:1256-1262.

Sindelar, J.J., Cordray, J.C., Sebranek, J.G., Olson, D.G., and Love, J.A. 2007. Investigating quality attributes and consumer acceptance of uncured, no nitrate/nitrite-added commercial hams, bacons and frankfurters. *J. Food Sci.* 72: S551-S559.

Laury, A. M., and Sebranek, J.G. 2007. Use of carbon monoxide combined with carbon dioxide for modified atmosphere packaging of pre-rigor and post-rigor fresh pork sausage. *J. Food Protect.* 70:937-942.

Bee, G., A. L. Anderson, S. M. Lonergan, and E. Huff-Lonergan. 2007. Extent of pH decline affects proteolysis of cytoskeletal proteins and water holding capacity in pork. *Meat Science* 76:359-365.

Lonergan, S. M., K. J. Stalder, T. J. Knight, E. Huff-Lonergan, R. N. Goodwin, K.J. Prusa, and D. C. Beitz. 2007. Influence of lipid content on pork sensory quality within pH classification. *Journal of Animal Science.* 85: 1074-1079.

Huff-Lonergan, E. and S.M. Lonergan. 2007. New frontiers in understanding drip loss in pork: Recent insights on the role of postmortem muscle biochemistry. *Journal of Animal Breeding and Genetics.* 124(Suppl.1):19-26.

Barbut, S., A.A. Sosnicki, S. M. Lonergan, T. Knapp, D. C. Ciobanu, L.J. Gatcliffe, E. Huff-Lonergan, and E.W. Wilson. 2007. Progress in reducing the pale, soft, and exudative (PSE) problem in pork and poultry meat. *Meat Science.* (In Press Online August 7, 2007. doi 10.1016/j.meatsci.2007.07.031).

Patton, B.S., C. Stahl and J.S. Dickson. 2007. On the role of Colicin E1 against gram-positive bacteria. *International Association for Food Protection Annual Meeting.* Orlando FL 7 - 11 July 2007.

Kudra, L.L., J.G. Sebranek, J.S. Dickson, A.F. Mendonca, K.J. Prusa, E. Larson, and J.C.Cordray. 2007. Control of *Listeria monocytogenes* on pre-cooked pork chops by irradiation combined with modified atmosphere packaging. *Reciprocal Meat Conference.* Brookings, SD. (June, 2007). Abstract no. 49, p. 28.

Kudra, L.L., J.G. Sebranek, J.S. Dickson and A.F. Mendonca. 2007. Control of *Escherichia coli* O157:H7 in ground beef patties with irradiation and modified atmosphere packaging. *Institute of Food Technologists Annual Meeting,* Chicago, IL. (July, 2007).

Participants:

D. G. Olson, J. G. Sebranek, S. M. Lonergan, J. S. Dickson

Target Audiences:

The target audiences of the information generated in this project include the professional meat science community (meat scientists in the meat industry and government institutions), who are responsible for the quality and safety of commercial meat products. Meat scientists at other academic institutions are also a target audience to provide critical review and comment on the work and to assist in communications and applications of the results of the work. Target audiences also include meat industry supervisory personnel who attend the extension short courses provided by Iowa State University and other academic institutions. The information generated by this project is further extended to the target audiences through scientific publications, extension publications and meat industry trade magazine articles.

Project Modifications:

None Reported

Approved (Signature)	Title	Date

U.S. Department of Agriculture Accomplishments Report AD-421 U.S. Dept. of Agriculture, State Agricultural Experiment Stations and Other Institutions			Date (Month, Day, Year) 03/05/2008
1. Accession 0202265	Agency Identification No. 2. CSREES 3. IOW	5. Work Unit/Project No. IOW05022	6. Status Final Report
7. Title The Conversion of Inactive Cobalamins to Coenzyme B12			
12. Investigator Name(s) (Last Name and Initials) Bobik, T. A.			
20. Termination Date 06/30/2007		40. Period Covered (mo/da/year): 10/01/2004 TO 06/30/2007	
Outputs: <p>The human ATP:cob(I)alamin adenosyltransferase (ATR) and methionine synthase reductase (MSR) enzymes are vital to human health because they are required for the conversion of vitamin B12 into its coenzyme forms, methyl-B12 (CH3-B12) and adenosyl-B12 (Ado-B12). One of the main goals of this research project is to characterize the human ATR and its interaction with MSR. Two common polymorphic variants of the human ATR (which catalyzes the terminal step in the conversion of vitamin B12 into Ado-B12) were produced in <i>Escherichia coli</i>, purified to apparent homogeneity and characterized biochemically. Analyses indicated that both variants had similar properties and were kinetically competent to mediate Ado-B12 synthesis in vivo. Further studies showed that MSR has cob(II)alamin reductase activity and can function in concert with the ATR enzyme for the conversion of cob(II)alamin to Ado-B12. Results indicated that the MSR and ATR enzymes specifically interact in such a way that a highly reactive reaction intermediate (cob(I)alamin) is sequestered. This is thought to be physiologically important for the prevention of nonspecific by-reactions by cob(I)alamin. Others previously showed that MSR functions in CH3-B12-dependent methionine synthesis. Findings obtained during this funding period indicate that MSR also functions as a cob(II)alamin reductase in support of Ado-B12 synthesis which raises the possibility that MSR could be a control point that regulates the flow of vitamin B12 to Ado-B12 and CH3-B12. To better define the biochemistry of B12 adenosyltransferase enzymes, we also conducted comparative studies on a bacterial enzyme from <i>Salmonella enterica</i>. Only the N-terminal domain of the bacterial enzyme was required for activity. NMR studies showed that this enzyme produced adenosine and triphosphosphate as its reaction products. Kinetic studies indicated a ternary complex mechanism rather than a substituted enzyme mechanism. A second major objective of the proposed studies is to construct gene therapy vectors useful for expression of human enzymes involved in inherited disorders of B12 metabolism. The human ATR cDNA was amplified by PCR and used to construct two recombinant adeno-associated virus vectors: rAAV1-ATR and rAAV2-ATR. Both vectors were produced in large amounts and delivered to C57/B16 mice. Eight weeks post-injection, liver and muscle were analyzed for the presence of vector and ATR by real-time PCR, western blotting and immunostaining. Liver contained between 0.03 and 0.1 vector copies per cell. Western blotting demonstrated the presence of ATR at levels 3-5-fold higher than the control. Thus, gene therapy vectors that express the ATR have been constructed. This is an important step toward treatment of certain B12-related diseases.</p>			
Outcomes/Impacts: <p>The conversion of vitamin B12 to CH3-B12 and Ado-B12 is essential to human health. Hereditary defects in this process are fatal in the first year of life in as many as 50% of cases and may account for some cases of sudden infant death syndrome. The metabolism of vitamin B12 is essential for recycling homocysteine into methionine and for maintaining cellular tetrahydrofolate pools. Thus, defects in vitamin B12 metabolism can lead to elevated homocysteine levels (a major risk factor in heart disease) as well as altered folate levels which is a potential risk factor in cancer. Further research on the genetics and biochemistry of B12 is needed to define the relationships between B12 and human disease and provide information useful for developing better methods for diagnosis, treatment and prevention of inherited disorders of B12 metabolism, and B12-related disease states. The construction of gene therapy vectors may lead to new treatments for diseases associated with rare inherited defects in B12 metabolism in humans.</p>			
Publications: Erger K.E., Conlon T.J., Leal N.A., Zori R., Bobik T.A., Flotte T.R. (2007) In vivo expression of human			

ATP:cob(I)alamin adenosyltransferase (ATR) using recombinant adeno-associated virus (rAAV) serotypes 2 and 8. *J. Gene Med.* 9,462-469

Sampson, E., Johnson, C. L. V. and Bobik, T. A. (2005) Biochemical evidence that the pduS gene encodes a cobalamin reductase. *Microbiology.* 151,1169-1177.

Kuhnl, J., Bobik, T. A., Procter, J., Burmeister, C., Hoppner, J., Wilde, I., Luersen, K., Torda, A., Walter, R., Liebau, E. (2005) Functional analysis of the methylmalonyl-CoA epimerase from *Caenorhabditis elegans* FEBS 272, 1465-1477.

Leal, N. A., Olteanu, H., Banerjee, R., and Bobik, T. A. (2004) Human ATP:Cob(I)alamin adenosyltransferase and its interaction with methionine synthase reductase. *J. Biol. Chem.* 279, 47536-47542.

Sheppard, D. E., Penrod, J. T., Bobik, T. A., Kofoid, E. and J.R. Roth. (2004) Evidence that a B12-adenosyl transferase is encoded within the ethanolamine operon of *Salmonella enterica*. *J. Bacteriol.* 186, 7635-7644.

Johnson, C. L. V., Buszko, M. L. and Bobik, T. A. (2004) Purification and initial biochemical characterization of the *Salmonella enterica* PduO ATP:cob(I)alamin adenosyltransferase. *J. Bacteriol.* 186, 7881-7887

Bobik, T.A, and Rasche, M.E. (2004) Purification and partial characterization of the *Pyrococcus horikoshii* methylmalonyl-CoA epimerase. *Applied Microbiology and Biotechnology.* 63, 682-685.

Participants:

Thomas A. Bobik PI directed the project. Training opportunities were provided for 3 graduate students. Celeste Johnson was able to obtain a PhD based on her work on this project. Nicole Leal obtained a PhD in part based on her work on this project. Edith Sampson obtained an MS degree partly based on her work on this project.

Target Audiences:

None Reported

Project Modifications:

None Reported

Approved (Signature)	Title	Date