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Abstract

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Grant Number:	1R01HD035687-01
PI Name:	STOVER, PATRICK J.
PI Email:	pjs13@cornell.edu
PI Title:	ASSOCIATE PROFESSOR
Project Title:	REGULATION OF FOLATE CATABOLISM

Abstract: DESCRIPTION: The catabolism of cellular folate is not well understood and has been assumed to be a non enzymatic process. However, a number of studies have shown that cellular folate catabolism is increased during periods of fetal development and as a result of chronic alcohol exposure which suggests that folate catabolism is regulated. The applicant has recently identified an enzyme in human cell lines that specifically cleaves 5-formyltetrahydrofolate (5-formylTHF) to p-aminobenzoylglutamate. Also, he has purified this enzyme from rat liver. He proposes to study the role of this 5-formylTHF hydrolyase in maintaining intracellular folate concentrations and the regulation of this enzyme. The specific aims are: 1) to clone and characterize the cDNA from both human and rat cDNA libraries and to overexpress, purify, and characterize the enzyme in both eukaryotic and prokaryotic systems; 2) the human gene will be cloned, sequenced, and its chromosomal location determined. The promoter region will be analyzed for consensus DNA regulatory elements and the contribution of the elements to regulation of expression will be determined; 3) cell culture models will be developed to study the role of 5-formylTHF catabolism in regulating intracellular folate concentrations by overexpressing the human 5-formylTHF hydrolyase gene in both the sense and antisense orientation. This will allow the effects of variable hydrolyase activity on folate accumulation and one-carbon metabolism to be determined; and 4) the role of the hydrolyase, cytoplasmic serine hydroxymethyltransferase (cSHMT), and 5,10-methenylTHF synthetase activities in the increased folate catabolism seen in pregnancy will be studied in a rat model using antibodies and cDNA probes to these enzymes in order to determine their level of transcription and expression in maternal and fetal tissues. These studies are expected to provide much new information about the regulation of folate catabolism.

Thesaurus Terms:
biotransformation, folate, genetic regulation
amine oxidoreductase, aminopterin, complementary DNA, enzyme activity, genetic regulatory element, leucovorin, nucleic acid sequence
DNA footprinting, gel mobility shift assay, laboratory rat, molecular cloning

Institution:	CORNELL UNIVERSITY ITHACA ITHACA, NY 14853
Fiscal Year:	1997
Department:	NUTRITIONAL SCIENCES
Project Start:	01-AUG-1997
Project End:	31-JUL-2002
ICD:	NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT
IRG:	NTN

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Grant Number:	5R29DK049621-03
PI Name:	STOVER, PATRICK J.
PI Email:	pjs13@cornell.edu
PI Title:	ASSOCIATE PROFESSOR
Project Title:	REGULATION OF SERINE HYDROXYMETHYLTRANSFERASE

Abstract: The metabolic roles of human cytosolic and mitochondrial serine hydroxymethyltransferase (SHMT) and the factors that contribute to their gene expression and catalytic activity will be determined using molecular biology techniques. The role of the SHMT catalyzed formation of 5- formyltetrahydrofolate, and the effects of -5-formyltetrahydrofolate accumulation on cellular metabolism and homeostasis will be investigated. The following approaches will be taken: l. The human SHMT genes will be cloned, sequenced and analyzed for consensus DNA regulatory elements. Consensus or novel cis-acting sequence elements that contribute to SHMT expression will be identified using chloramphenicol acetyltransferase assays and in vivo footprint analysis. The associated trans-acting factors will be identified and characterized by gel-mobility shift assays and southwestern analysis. 2 The mechanisms through which external stimuli regulate SHMT expression will be elucidated. Cultured cells will be treated with stimuli that are known to influence SHMT activity. The effects of these stimuli on SHMT mRNA levels and synthesis rates will be determined using a reverse transcriptase-polymerase chain reaction assay and nuclear runoff assays. Changes in mRNA will be compared to SHMT enzyme activity. The effects of external stimuli on folate one-carbon pools, intracellular serine/glycine concentrations and on the 5-formyltetrahydrofolate futile cycle will also be determined by HPLC analysis. 3. The role of the 5-formyltetrahydrofolate futile cycle in mammalian cellular homeostasis will be studied. Cell culture models will be developed where the futile cycle is manipulated by methenyltetrahydrofolate synthetase inhibitors, varying intracellular serine/glycine ratios and by disrupting and/or over expressing SHMT and methenyltetrahydrofolate synthetase. The effects of 5- formyltetrahydrofolate accumulation on cellular glycine concentrations, one-carbon metabolism, global protein synthesis, and gene transcription in mammalian cells will be investigated. The long term goal of these studies is to (l) differentiate the metabolic roles of mitochondrial and cytosolic SHMT and determine if their regulation influences the supply of folate-activated one-carbon units, (2) determine how 5-formyltetrahydrofolate concentrations are regulated in mammalian cells, (3) define the role of the SHMT catalyzed formation of 5-formyltetrahydrofolate in influencing cell metabolism and proliferation.

Thesaurus Terms:
enzyme activity, gene induction /repression, hydroxymethyltransferase, serine, structural gene
chloramphenicol acetyltransferase, cytokine, cytoplasm, folate, genetic model, genetic regulatory element, glycine, growth factor, isozyme, mitochondria, model design /development, steroid hormone, tetrahydrofolate, transcription factor
DNA footprinting, high performance liquid chromatography, molecular cloning, nuclear runoff assay, polymerase chain reaction, tissue /cell culture

Institution:	CORNELL UNIVERSITY ITHACA ITHACA, NY 14853
Fiscal Year:	1997
Department:	NUTRITIONAL SCIENCES
Project Start:	01-AUG-1995
Project End:	31-JUL-2000
ICD:	NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES
IRG:	ZRG4