improvements in our understanding of the exact site and mechanism of the biotransformation of organic nitrates, including a better understanding of the biochemical intermediaries involved, may well lead to a better understanding of the cause of this phenomena.

**Sustained therapy with organic nitrates: is it safe?**
The concept that sustained nitrate therapy causes increased production of free radicals has a number of important implications. Although their clinical efficacy during chronic therapy had been questioned for many years because of phenomena of tolerance, it had generally been assumed that the organic nitrates were safe drugs with no potential for long-term toxicity. Some authors proposed that organic nitrates, as exogenous NO donors, would be beneficial in the setting of chronic atherosclerosis, as they could compensate for the decrease in NO bioavailability that characterizes that disease state. Although the hypothesis was raised, it has never been tested in a long-term trial of clinical outcomes. Today, multiple lines of evidence suggest that this assumption of safety is likely to be incorrect. A series of human observations have documented that therapy with nitroglycerin causes severe abnormalities in endothelial function, as defined by abnormalities in the response to endothelium-dependent vasodilators, in both the coronary and peripheral arterial circulation (reviewed in refs. 2 and 3). The present study (8), remarkably, documents that therapy with nitroglycerin causes increased free radical production by cardiac mitochondria as well as abnormalities in aortic ALDH-2 activity. These observations suggest that therapy with nitroglycerin can cause important abnormalities in mitochondrial respiration and oxygen consumption. Although the observation that this occurs in cardiac mitochondria is somewhat surprising, it again raises questions concerning the safety of these agents in the long-term therapy of cardiac disease. Taken together, these observations suggest that the time has come to mount an appropriately powered clinical trial examining the efficacy and safety of sustained therapy with organic nitrates for those diseases for which they are commonly used.


**Tandem mass spectrometry in discovery of disorders of the metabolome**

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Genetic disorders of amino acid and fatty acid metabolism can be detected with tandem mass spectrometry (MS/MS). MS/MS screening of mice subjected to chemical mutagenesis (see the related article beginning on page 434) defined a new disorder of branched-chain amino acid metabolism resembling human maple syrup urine disease. This approach has general application to the discovery of gene function in developmental and metabolic disorders.


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**Nonstandard abbreviations used:** tandem mass spectrometry (MS/MS); maple syrup urine disease (MSUD).

The post-genomic era has arrived and has created an exciting environment for the discovery of genetic disorders, multicentric etiologies of disease, and pharmacogenomics, and the delineation of gene function, with unusual approaches (1). In this issue of the *JCI*, Y.-T. Chen, an accomplished biochemical geneticist, and coworkers use an intriguing process with potential general application to mammalian disease and congenital anomalies to demonstrate the phenotype of a new metabolic disorder (Figure 1). The results document the usefulness of studying the “metabolome,” a term that refers to the low-molecular-weight compounds present in organisms that are substrates, products, and regulatory molecules in macromolecular metabolic pathways. This commentary projects three major conclusions. First, screening of mutagenized animals with clinically relevant approaches will define phenotypes similar to critical human disorders. Second, the study of the metabolome will likely define critical compounds, relevant genetic disorders, and modifying genes in animal models that will be revealing in our understanding of human disease. Third, highly sensitive technologies applied to both animal models and humans will result in the discovery of new disorders; the aberrant, causative genes; and new treatments.

**Metabolomics, screening, and tandem mass spectrometry**

Following chemical mutagenesis of mice using N-ethyl-N-nitrosourea and...
The application of typical medically relevant clinical and laboratory assessment of large populations of mutagenized animals, especially those such as mice and zebrafish that have relatively short reproductive cycles, gestations, and life spans, and adequately characterized genomes, opens up an entirely new era for the characterization of human anomalies and disease. In zebrafish, large-scale screens for developmental anomalies of the cardiovascular (3) and other organ systems through visual observation have allowed delineation and characterization of genes essential for organogenesis that have human homologs and are mutated in human disease. In mice, screening for phenotypes highly relevant to common human disorders, including such examples as hypertension, diabetes, hyperlipidemias, congenital anomalies such as congenital heart disease, and behavioral or movement disorders, is now possible with three generations of breeding designed to assess recessively inherited disorders, the authors screened families of mutant mice for disorders of amino acid and fatty acid metabolism using tandem mass spectrometry (MS/MS). MS/MS analysis of body fluids, including plasma, urine, bile, and tissues has become a powerful tool to discern some 40 metabolic disorders in humans and is now the method of choice for newborn screening of these disorders in many American states and European countries (2). In this instance (1), elevations of branched-chain amino acids in one family of mutagenized mice suggested a disorder similar to human maple syrup urine disease (MSUD), a disorder that is secondary to mutations in the branched-chain α-keto-acid dehydrogenase complex. However, in the murine family, a missense mutation in another enzyme in this amino acid pathway, the mitochondrial branched-chain aminotransferase, was discovered. Thus, through the use of mutagenesis of mice and metabolome screening of offspring with MS/MS, a novel disorder with a phenotype similar to a known human metabolic disease was discovered. Because mouse and human metabolism is similar, it is very likely that humans with a similar clinical and biochemical phenotype will turn out to have mutations in this gene. Thus, use of the highly sophisticated methodology of MS/MS, currently applied to human newborn screening, in mice led to the discovery of a murine genetic disorder that in all probability will subsequently be found in people.

**Aminotransferase**

Three generations of breeding designed to assess recessively inherited disorders, the authors screened families of mutant mice for disorders of amino acid and fatty acid metabolism using tandem mass spectrometry (MS/MS). MS/MS analysis of body fluids, including plasma, urine, bile, and tissues has become a powerful tool to discern some 40 metabolic disorders in humans and is now the method of choice for newborn screening of these disorders in many American states and European countries (2). In this instance (1), elevations of branched-chain amino acids in one family of mutagenized mice suggested a disorder similar to human maple syrup urine disease (MSUD), a disorder that is secondary to mutations in the branched-chain α-keto-acid dehydrogenase complex. However, in the murine family, a missense mutation in another enzyme in this amino acid pathway, the mitochondrial branched-chain aminotransferase, was discovered. Thus, through the use of mutagenesis of mice and metabolome screening of offspring with MS/MS, a novel disorder with a phenotype similar to a known human metabolic disease was discovered. Because mouse and human metabolism is similar, it is very likely that humans with a similar clinical and biochemical phenotype will turn out to have mutations in this gene. Thus, use of the highly sophisticated methodology of MS/MS, currently applied to human newborn screening, in mice led to the discovery of a murine genetic disorder that in all probability will subsequently be found in people.

**Animal models of metabolic disorders**

Chen and coworkers (1) utilized their newly discovered model of human MSUD to assess the efficacy of dietary management used in humans. The mutant mice exhibited failure to thrive, muscular weakness, hair loss, and premature death. All of these features were reversed by feeding the animals a diet low in branched-chain amino acids. This result demonstrates the important general principle that murine mammalian models of human disorders can be used to determine the natural history of such disorders and quickly assess outcomes of treatment, studies that are often impossible to complete in humans and, even if feasible, take years to perform. Although gene ablation mouse models have allowed similar studies, knockout mice that are null for gene expression often are embryonic lethal or have a much more severe phenotype than those of monogenic human disorders that are secondary to missense or splice site mutations with partial expression or activities of protein. Thus, as illustrated by this manuscript (1), a milder phenotype more typical of many human genetic disorders is often more relevant to human disease than a complete knockout. Another obvious advantage of these animal models is the ability to study outcomes in the whole organism, not just in cell lines derived from human patients.

**Animal model screening**

The application of typical medically relevant clinical and laboratory assessment of large populations of mutagenized animals, especially those such as mice and zebrafish that have relatively short reproductive cycles, gestations, and life spans, and adequately characterized genomes, opens up an entirely new era for the characterization of human anomalies and disease. In zebrafish, large-scale screens for developmental anomalies of the cardiovascular (3) and other organ systems through visual observation have allowed delineation and characterization of genes essential for organogenesis that have human homologs and are mutated in human disease. In mice, screening for phenotypes highly relevant to common human disorders, including such examples as hypertension, diabetes, hyperlipidemias, congenital anomalies such as congenital heart disease, and behavioral or movement disorders, is now possible with three generations of breeding designed to assess recessively inherited disorders, the authors screened families of mutant mice for disorders of amino acid and fatty acid metabolism using tandem mass spectrometry (MS/MS). MS/MS analysis of body fluids, including plasma, urine, bile, and tissues has become a powerful tool to discern some 40 metabolic disorders in humans and is now the method of choice for newborn screening of these disorders in many American states and European countries (2). In this instance (1), elevations of branched-chain amino acids in one family of mutagenized mice suggested a disorder similar to human maple syrup urine disease (MSUD), a disorder that is secondary to mutations in the branched-chain α-keto-acid dehydrogenase complex. However, in the murine family, a missense mutation in another enzyme in this amino acid pathway, the mitochondrial branched-chain aminotransferase, was discovered. Thus, through the use of mutagenesis of mice and metabolome screening of offspring with MS/MS, a novel disorder with a phenotype similar to a known human metabolic disease was discovered. Because mouse and human metabolism is similar, it is very likely that humans with a similar clinical and biochemical phenotype will turn out to have mutations in this gene. Thus, use of the highly sophisticated methodology of MS/MS, currently applied to human newborn screening, in mice led to the discovery of a murine genetic disorder that in all probability will subsequently be found in people.
newer or microtechnologies for the measurement of blood pressure, blood clinical chemistries, echocardiography, and behavioral screening.

**Conclusion**

More widespread application of the mutagenesis approach reported (1) with screening by clinical observational and sophisticated laboratory studies will allow delineation of a broader spectrum of genetically determined or modified disorders directly relevant to human disease.