Dr. Fernstrom initiated the discussion with a question regarding how the body handles the additional S-adenosylmethionine (SAM) generated when dietary methionine intake is increased. Does the excess SAM stimulate methylation reactions? Dr. Mudd responded that when excess SAM is generated by an increase in dietary methionine intake, the flux through glycine N-methyltransferase (GNMT) increases, which shunts the excess SAM into N-methylglycine (sarcosine) and subsequently methylene tetrahydrofolate and glycine. Dr. Brosnan added that the methylene tetrahydrofolate ultimately loses the single carbon via mitochondrial pathways to carbon dioxide. Dr. Fernstrom asked if that meant that the excess methylene tetrahydrofolate drove no excessive methylation. Dr. Brosnan replied that a rise in SAM concentration might constitute a “steady-state pressure” on methylation (e.g., DNA methylation) but that GNMT activation by SAM would limit any such possibility. Dr. Selhub added that in tissues other than liver, SAM concentrations appear not to vary with dietary methionine intake. The liver is the principal locus of methionine metabolism in the body, the main site where SAM concentrations rise when a high-methionine meal is ingested, and contains a high activity of GNMT. It is thus the focus of SAM elimination in response to a methionine load. He also noted that explanations for adverse effects of high methionine levels are not really known. For example, the exposure of mouse embryos to high methionine concentrations in vitro was reported to induce neural tube defects; because SAM synthesis would be limited, a mechanism involving SAM would be unlikely. Currently, a biochemical mechanism is lacking. Dr. Mudd added that MAT1A, the gene encoding the active subunit of methionine adenosyltransferases I (MAT I) and III (MAT III), the isoforms of MAT that predominate in the adult liver, is not expressed in the fetus. MAT1A is expressed in liver only postpartum. In the embryo, SAM synthesis would be carried out by MAT II, the isoform encoded by gene MAT2A.

Dr. Smith then asked the fate of excess methionine in tissues other than liver, notably brain, where SAM synthesis is not stimulated by high methionine concentrations, and thus no apparent mechanism exists for its elimination. He wondered what the impact of high methionine concentrations might be and if toxic endpoints might be identified and studied. He further noted that differences in the metabolic handling of methionine among organs (e.g., the relative deficiency in transsulfuration in brain) might require careful examination of each organ.

Dr. Mudd commented that the brain would be metabolically complicated; for example, it synthesizes SAM using MAT II and uses it to methylate a variety of biologically active molecules, such as neurotransmitters. He also noted his experience in human subjects bearing mutations in MATI (deficient in MAT I/III activity): some individuals with homozygous MATI truncating lesions develop demyelination of the brain. In I such patient, CSF methionine concentrations were abnormally high (because of hypermethioninemia). However, despite the fact that brain MAT II activity was presumably normal (its activity was normal in leukocytes and fibroblasts), CSF SAM concentrations were abnormally high, suggesting that normally, SAM may be delivered from liver to brain. Finally, he added that the human brain lacks cystathionine γ-lyase activity and therefore accumulates cystathionine; how the brain eliminates cystathionine is presently unknown. Hence, it is possible that excess methionine ingestion might promote cystathionine accumulation in the brain, with unknown consequences.

Dr. Rees noted that cell and embryo culture experiments often employ very high concentrations of methionine, very much higher than physiological levels, and this fact should be considered in interpreting such studies.

Dr. Finkelstein added that the discussion highlighted several important and unresolved questions. First, an acute excess of methionine does influence methionine metabolism in extrahepatic tissues. For example, in studies in which the administration of methionine greatly elevates hepatic SAM production, renal S-adenosylhomocysteine (SAH) doubles, although renal SAM is unchanged. How does this occur? Second, as Dr. Smith noted, the brain does not have a...
transsulfuration pathway, in the strictest sense, but does have cystathionine synthase. What is all that cystathionine doing in the brain? And third, Dr. Rees’ comment raises an important topic, that of experimental artifacts. What models should we be using? Dr. Rees expressed concern about excessive concentrations of methionine; the same concerns exist for many, if not most, in vitro studies of homocysteine metabolism and toxicity. We must be careful with our models because the resultant data, although interesting, may be leading us away from meaningful physiological understanding.

Dr. Baker then asked if anyone knew the extent to which, under conditions of folate/B-12 deficiency, betaine-homocysteine methyltransferase (BHMT) could remethylate homocysteine, or, under conditions of choline/betaine deficiency, methionine synthase could remethylate homocysteine? Dr. Selhub responded that if homocysteine were the issue, betaine would be useful but probably would not normalize homocysteine in tissues other than the liver that lack betaine-homocysteine methyltransferase. He then added that tissue compartmentalization of the l-carbon cycle was important to consider in this regard. For example, in animals maintained on a folate-deficient diet, all tissues become folate deficient except for brain. And in brain, homocysteine levels are low, not high, and it is thus difficult to reconcile this fact with the hypothesis that cognitive impairment associated with folate/B-12 deficiency relates to elevated homocysteine concentrations in brain.

Dr. Fernstrom then asked, in relation to Dr. Baker’s comment, if there were variations in the diet that might occur normally that would alter substrates or cofactors and cause meaningful differences in the pathways we are discussing? Otherwise, it seemed to him, the discussion was simply an intellectual exercise about animals in a cage getting an aberrant diet. Dr. M. Brosnan noted that because methionine enters the brain on the large neutral amino acid transporter, which is competitive, raising plasma methionine concentrations by an increase in dietary methionine intake could block the transport into brain of amino acids that are precursors for neurotransmitters (tryptophan, tyrosine) and thus adversely affect brain function. Dr. Finkelstein reflected that the question raised by Dr. Baker brings to mind the experiments of DuVigneaud and of Bennett. DuVigneaud showed that homocysteine and choline/betaine could (or were presumed to be able to) totally replace methionine in the diet. He noted that, in retrospect, this cannot have worked; a folate contaminant must have been present. Bennett showed that animals maintained on a marginal methionine ration died without choline and betaine but thrived when provided with (what we now know is) folic acid and cobalamin. He added that human genetic disorders provide additional examples. For example, in animals maintained on a folate-deficient diet, all tissues become folate deficient except for brain. And in brain, homocysteine levels are low, not high, and it is thus difficult to reconcile this fact with the hypothesis that cognitive impairment associated with folate/B-12 deficiency relates to elevated homocysteine concentrations in brain.

Dr. Ball then directed a question to Dr. Baker regarding an amino acid he had mentioned, S-methylmethionine, and inquired if it is a methyl donor and, if so, could it be used to replace choline or betaine and provide a methyl group, which then ultimately could spare methionine? He noted that S-methylmethionine is present in the normal diet as a plant-derived amino acid. Dr. Baker responded first that in addition to S-methylmethionine, other methyl compounds occur in the human food supply, such as dimethyl sulfonyl propionate, present in algae (some humans consume significant amounts of algae). He then noted that S-methylmethionine has methylating capacity and can spare choline, as demonstrated in rats and chicks, and further that his group will soon begin examining if dimethyl sulfonyl propionate can do the same thing. Dr. Ball responded that the implication would be, then, that if S-methylmethionine is absorbed in the gut, it could be involved in many other methylation reactions. Dr. Baker agreed, noting that it could methylate either directly or indirectly, through the biosynthesis of SAM, because it might replace betaine in the BHMT reaction. Dr. Finkelstein asked if it could replace choline/betaine, to which Dr. Baker replied in the affirmative, noting that S-methylmethionine had once been labeled a “vitamin” (the so-called vitamin U). Dr. Mudd added that S-methylmethionine could be of only limited utility, however, because its ingestion leads to the formation of dimethylsulfide, a volatile, unpleasant-smelling compound, and thus would produce bad breath.

Dr. Brosnan then turned back to the question of the extent to which betaine could substitute for dietary methyl groups in maintaining methylation reactions. He felt it important that choline and betaine were components of the diet, and thus the extent to which they could be used depended largely on their levels in the diet. In this regard, he noted that although it is true that the body can make choline via phosphatidylethanolamine methyltransferase (PEMT), it costs three methyl groups to do so, so that this pathway does not represent a net increase in available methyl groups. Hence, he observed that the extent to which betaine could be used in the betaine:homocysteine methyltransferase reaction is largely a function of dietary betaine and choline contents. Dr. Mudd added that experimental animals survive on a methionine-free, homocysteine-supplemented diet if given enough betaine. They can also form sufficient methionine from homocysteine if provided with adequate folate and B-12.

Dr. Baker noted that Dr. Brosnan had made a quantitative assessment of the pathways that consume SAM methyl groups and wondered if Dr. Brosnan would care to discuss these calculations. He commented that he had thought that creatinine synthesis consumed most SAM methyl groups and wondered if this was still a correct perception. Dr. Brosnan confirmed that the work of Mudd and Poole (and that of others) indeed suggested that creatinine synthesis required a SAM methyl group and that a large amount of creatine was synthesized daily by the body, at least as indicated by the amount of creatinine excreted each day. But he suggested that the pathway may not consume as much of the total SAM pool as originally described because original calculations were based on creatinine excretion, not creatinine synthesis. Because dietary creatine (from meat) contributes to body creatine pools and thus to creatinine excretion, a recalculation shows that creatine synthesis still makes a very major demand on SAM, but less than the 70% figure originally proposed. What might other metabolic sinks for SAM be? There may be many small ones, and also perhaps a big one: On the basis of experiments with PEMT knockout mice, his group found that PEMT (which synthesizes phosphatidyl-choline) might also be a dominant user of SAM. He noted that
there are no direct data in humans, but certainly in mice it seems that PEMT makes an appreciable demand on SAM pools. Hence, although creatine synthesis is a very important user of SAM, it is not as dominant as we once thought.

Dr. Mudd agreed about this assessment of creatine synthesis, noting that there is a significant amount of creatine in the American diet. To the list of pathways that are major SAM users he then added GNMT, commenting that children who lack this enzyme accumulate very high amounts of SAM and methionine. This fact suggests that normally, glycine methylation consumes considerable SAM. He further noted from his own work that when excess methionine is fed to saccrine dehydrogenase-deficient subjects, saccrine excretion rises after methyl intake exceeds that needed to synthesize creatine and other necessary compounds and could account for the excess methionine intake. He commented that the kinetic properties of GNMT (positive cooperativity with respect to SAM; little inhibition by S-adenosylhomocysteine; inhibition by methylytetrahydrofolate) made this enzyme well adapted for such a role. Dr. Finkelstein added that he thought that most people consume methionine in excess, and that this fact thrust GNMT to the forefront as a SAM consumer. The difference between GNMT and guanidinoacetate methyltransferase or phosphatidylethanolamine methyltransferase, for example, is that the latter two enzymes are essentially constitutive, whereas GNMT eliminates the excess SAM. He agreed with Dr. Mudd that GNMT is probably the dominant methyltransferase but that this state reflects dietary excess (of methionine intake), not an actual biologic need for the product of the enzymatic reaction.

Dr. Waterland then asked Dr. Brosnan to comment further on dietary creatine and SAM, especially in the context of infants and the creatine content of infant formulas. Dr. Brosnan offered some new observations. Inborn errors of the two enzymes of creatine synthesis have been identified, as well as of the creatine transporter. In all cases, neurological problems were present, such as epilepsy, speech delay, and mental retardation. Thus, he noted, it appears that there is something about creatine synthesis that is important for brain development (but about which nothing is presently known). He then commented that he and his associates had recently measured the creatine content of several infant formulas. Cow’s milk-based formulas were found to contain substantial creatine, whereas soy-based formulas contained none. He noted that about 25% of North American children ingest soy-based formulas, a fact that now bears reflection because creatine appears to be important in brain development. Dr. Mudd added that guanidinoacetate methyltransferase-deficient children who do develop neurological problems benefit greatly from creatine feeding (though not completely: they continue to have some clinical problems).

Dr. Fernstrom then asked Dr. Stipanuk if one could conclude from her talk that there is regulation or control of cysteine pools in the body, to which she replied in the affirmative. He then asked if the body cysteine pool is “buffered” from variations in dietary cysteine intake. Dr. Stipanuk again responded in the affirmative, noting that it is probably buffered completely within a range of intakes. She commented that, as dietary sulfur amino acid levels rise, degradation capacity increased markedly, a response that would certainly keep body and tissue cysteine levels from rising as high as they otherwise might. She noted, for example, that in intact animals fed a 10% casein diet (with no added sulfur amino acids), a level low enough for the sulfur amino acid intake to limit growth, hepatic cysteine dioxygenase (CDO) activity is almost undetectable, and hepatic cysteine concentrations are about 20 nmol/g. But if animals are fed a 40% casein diet (with no methionine supplementation), and now sulfur amino acid intake is well above requirement, hepatic CDO activity is markedly higher than that in animals ingesting 10% casein. The hepatic cysteine concentration rises to 80–100 nmol/g, 4–5 times that in animals fed 10% casein. But, she added, it would no doubt have risen much higher without the increase in enzyme activity. She further added that CDO is not expressed in most tissues and that the rise in CDO activity occurs only in liver, making the liver responsible for buffering the flow of cysteine into the body. She then noted that some increase also occurs in the shunting of cysteine to glutathione when sulfur amino acid intake rises, which may also help to remove excess cysteine (glutathione levels essentially mirror cysteine levels in the tissues, indicating that under normal conditions, cysteine is limiting for glutathione synthesis). Dr. Fernstrom asked if the effect on glutathione production occurs in liver. Dr. Stipanuk replied that liver is the focus and that much of the sulfur amino acid flux into liver is converted to glutathione and exported as glutathione. Dr. Fernstrom asked how fast glutathione synthesis rises in response to increased sulfur amino acid ingestion. Dr. Stipanuk replied that the changes occur rapidly, within hours, based on recent data from her laboratory.

Dr. Fernstrom then asked if anyone would be willing to comment on the inability of brain to synthesize cysteine from methionine and, more generally, on the handling of cysteine by brain, including its uptake from blood as cystine and the cellular compartmentation of glutathione production in brain. Dr. Stipanuk offered the comment that the metabolism of sulfur amino acids in brain is not well understood. She indicated that she was uncertain if there is no transsulfuration in brain. She felt that metabolism probably does differ between neurons and glia, but knowledge is limited. Moreover, the usefulness of results obtained from studies of cell cultures (such as neurons and glia) may be limited in terms of their applicability in vivo because, in general, cultured cells rapidly up-regulate the cysteine transporter and begin to take up cystine. As an example, she noted from work in her laboratory that cultured liver cells (HepG2) up-regulate the cysteine transporter and develop high intracellular cysteine levels and do not express CDO or liver-specific methionine adenosyl transferase. They thus do not really look that much like liver cells in vivo. Even cultured primary rat hepatocytes rapidly begin to down-regulate CDO expression and up-regulate cysteine transport once placed in culture. Dr. Smith added that from some of his early work, he had observed that pyridoxine-deficient rats had 25% lower glutathione levels in brain. He conducted the experiment to assess if there was transsulfuration in brain. He noted that one way to interpret decreased glutathione levels in the brains of pyridoxine-deficient rats was that some transsulfuration was occurring. He added that there was a very high level of cystathionine in the brains of pyridoxine-deficient rats, showing that at least that part of the pathway was viable. Dr. Mudd added that he thought there might be a species-specific difference in the ability to carry out transsulfuration in the brain and that humans and primates have unusually high cystathionine concentrations attributable to the lack of cystathionine γ-lyase in their brains. He thought that other species did not have similarly high cystathionine brain concentrations but was uncertain where the line between species fell.

Dr. Baker then changed direction, asking in relation to homocysteinemia, 1) what concentrations of homocysteine in blood were considered too high, and 2) for people who eat diets high in animal protein (especially organ meats), to what extent are they consuming homocysteine? Dr. Refsum took up this issue, commenting that she would cover this point in some detail in the talk she would present but that the short answer to
the first question was that “we do not know.” She noted that in certain inborn errors of metabolism, where homocysteine levels were extremely high, the condition was not healthy, but that in the general population, no firm evidence existed as to what level is too high. Concerning the second question, the answer was also unknown at present. She noted that there is no clear evidence that, for example, the intake of meat leads immediately to increased homocysteine levels; the rise appears to be delayed, in such a manner as to suggest that the increase might result from increased methionine metabolism. But whether there is homocysteine in meat products, she added, of course you would expect some, but not very much, because intracellular homocysteine concentrations are lower than in plasma. Moreover, she added, even if the diet contained homocysteine, and it were absorbed, she would expect much of it to be cleared by the liver and not reach the general circulation.

Dr. Fernstrom asked if someone would comment about the mention of cysteamine in one of the earlier talks and its inclusion in a dietary study because this agent has been found to be an ulcerogen and also to suppress the biosynthesis of some peptides in brain. Dr. Stipanuk responded that cysteamine is a normal metabolite of coenzyme A (CoA). As soon as cysteine is incorporated into the CoA precursor 4-phosphopantetheo-cysteine, it is immediately decarboxylated. Hence, when CoA is ultimately degraded, instead of cysteine, cysteamine (decarboxylated cysteine) is released. And there is, in fact, a cysteamine dioxygenase in tissues that can oxidize cysteamine to hypotaurine. She commented that she presumed cysteamine dioxygenase is analogous to cysteine dioxygenase, although the enzyme and gene have not been identified; both enzymes oxidize the sulphydryl group of its substrate to a sulfinic acid group. She added that probably some cysteamine is produced and serves as a precursor for taurine, but the question is how much? Is this a minor or a major pathway? She noted that effects of cysteamine had been demonstrated in pharmacological studies and that many were produced by the disulfide form cystaine, which reacts with protein thiol groups (to oxidize them) and thus produces its effects. Cysteamine, the sulphydryl form, being a fairly potent antioxidant, may have some protective effects. But she commented that she did not know if there were differences, depending on whether cysteamine or cystamine, the thiol or the disulfide, was administered. Dr. Fernstrom responded that from the study in the earlier talk, cysteamine had been included in the diet at 7.2 g/kg, which would give a 300-g rat a daily dose of around 700 mg/kg. This is a fairly good sized dose, he noted, given that pharmacologic studies had used 30–300 mg/kg cysteine in rats (as a single dose), and he wondered if gastrointestinal, endocrine, and brain effects might not be present in such dietary studies, independent of any metabolic effects. Dr. Stipanuk responded that such might well be the case, and also that some of the effects seen in pharmacologic studies might follow from cysteamine conversion to hypotaurine and taurine.

Dr. Selhub then asked Dr. Baker if he would indicate the structure of ketomethionine. Dr. Baker responded that keto-methionine is methionine with the α-amino group replaced by a keto group. Dr. Selhub asked if it has the same toxicity as methionine. Dr. Baker stated that he did not think this interesting question had been explored but that the use of ketomethionine in the diet (e.g., as a supplement) might possibly offer some advantages over methionine because methionine imparts a bad cabbage-like odor and flavor. He thought that α-keto methionine might not have this drawback because the α-amino group is involved, along with the sulfur group, in the Strecker degradation reaction that causes the flavor and odor problems humans dislike. He also noted that, without the α-amino group, it would not be subject to heat-induced Maillard loss of bioactivity. Dr. Selhub commented that he asked the question because he is attempting to develop a model involving the addition of methionine to drinking water to induce cardiovascular disease. However, the animals do not like the smell and taste and do not consume sufficient amounts (of methionine or water). The use of α-keto methionine as a methionine surrogate might be useful if it lacked the sensory properties of methionine. Dr. Baker thought that ketomethionine should be converted to L-methionine in animals. Dr. Mudd added the caution that although ketomethionine could be transaminated to L-methionine, it is also the first step in the transamination pathway for degrading methionine. That pathway gives rise to further metabolites that have possibly been implicated in methionine toxicity and to dimethylsulfide, a cause of malodorous breath.

Dr. Cynober commented that he thought that keto methionine had been used together with a mixture of other keto analogues, especially of the branched-chain amino acids, in the treatment of chronic renal failure (between 1970 and 1990). He said that it appeared to be an elegant way to recycle and use ammonia and that he did not remember any evidence of toxic effects. It might be useful to look at this literature. Dr. Baker agreed but noted that in the mixtures used in chronic renal patients, the hydroxy analogue, not the keto analogue of methionine is employed.Apparently, in humans, the hydroxy analogue is very active as a methionine precursor.

Dr. Fukagawa then asked if anyone would like to comment on the effects of ingesting genetically modified proteins, where the ratios of the sulfur amino acids may differ from those present in the natural proteins. She asked if there are human food sources that have been altered genetically to produce different ratios of methionine to cysteine, or of total sulfur amino acid content. Dr. Baker responded that there is a good deal of ongoing research in the area of transgenics and genetically modified cereal grains, oil seeds, and so forth. He was not aware if much attention was being given to increasing methionine. He noted that a strong focus has been on lysine because it is the most deficient amino acid in animal diets (though for poultry, methionine is the most limiting amino acid). Dr. Mudd added that he was aware of ongoing work to try to raise the methionine content of plants, especially in proteins. Dr. Ball commented regarding the enrichment of specific amino acids in plant proteins that a big stumbling block is that seed-producing plants (generally what we eat) produce seeds containing important functional proteins. Changing the primary structure of seed proteins can compromise the quality of the seed by modifying its ability to germinate and grow. He noted that several investigators in his department actively work on pulse grains (e.g., peas), and it is proving extremely difficult to make very much change in the amino acid profiles of their proteins without imparting bad agronomic characteristics: poor disease resistance, poor growth, and poor yield.

Dr. Ball then raised a new question for discussion. He noted that during Dr. Waterland’s presentation on methylation of DNA and epigenetics, it occurred to him that the findings might link closely with Dr. Rees’ presentation on maternal programming of the fetus. He wondered if there are any data showing an increase in methylation in the fetus as a result of feeding higher levels of sulfur amino acids to the mother during gestation, and if so, what effect that might have on subsequent pre- and postnatal gene expression? Dr. Rees responded that his evidence and that from one other study might be the only studies showing changes in methylation. He noted that many of the genes examined in his study, particularly those related to tissue differentiation, were actually all regulated by methylation. This was partly why he
looked at that panel of genes. The evidence was, at least at the mRNA level, that no significant changes in expression were seen. Dr. Finkelstein commented that a possible source of data in humans might be women who have cystathionine synthase deficiency and have given birth to children. He wondered if they might be expected to have hypermethioninemia during pregnancy, and if so, was there was any evidence of fetal damage? Dr. Mudd responded that he thought there was no convincing evidence in this regard. He noted that normal children have been born to these women, though there has also been some fetal loss. However, he did not know if there might have been reporting bias (and to what extent) in the sense that pregnancies in women with cystathionine β-synthase (CBS) deficiency might have been reported more often in cases where trouble developed and less often in cases of normal gestational development and delivery. He added, though, that when his group examined the outcomes of pregnancies of women with CBS deficiency, they did not find a big increase in birth defects.