Review

Aspartame consumption: lack of effects on neural function

Abel Lajtha, Margaret A. Reilly, and David S. Dunlop

The Nathan S. Kline Institute for Psychiatric Research, Center for Neurochemistry, Orangeburg, NY USA

Keywords: aspartame; aspartic acid from aspartame; phenylalanine from aspartame; amino acids and aspartame; phenylketonuria; seizures and aspartame; neurotransmitters and aspartame

Introduction

Food additives have rarely been examined using neurochemical methodology for their effect on the nervous system. Often the blood-brain barrier was assumed to provide impenetrable protection, and effects on the brain were undetectable from animal behavior or brain histopathology studies. However, direct or indirect influences of nutritional elements on processes in the central nervous system can be significant, although sometimes subtle, just as nutritional deficiencies can have significant effects. Aspartame is unusual in that its possible central nervous system effects have been studied extensively–all food additives should undergo such rigorous testing. The recent studies are reviewed here.

Aspartame (1-aspartyl-1-phenylalanine methyl ester) is a sweetener that is now in wide and frequent use. It was discovered by chance over 25 years ago and was first used as a food additive 13 years ago. Numerous studies have been made on various aspects of this compound, and the extensive literature includes observations on its absorption, metabolism, taste, structural analogs, effects on nutrition, etc. Much of the literature was summarized and discussed in the book *Aspartame,*[1] published in 1984. Because of the continuing interest and the large number of reports, additional reviews of aspartame studies have been published,[2–6] and a recent book discusses many aspects of the effects of phenylalanine (including aspartame-derived phenylalanine) on the nervous system.[7] Several reviewers have raised concerns in regard to potential or anecdotal interactions.[8–11] Although brain function is well protected from the influence of nutritional variations, as demonstrated by the tolerance of the brain to the diversity of the human diet, it has become increasingly evident that it is necessary to examine the effects of food constituents or additives. In the case of aspartame, because it gives rise to aspartic acid, phenylalanine, and methanol, each of which may have effects on the nervous system at high blood levels, several aspects need to be considered.

The present review incorporates recent studies of possible effects of aspartame on the brain into our presently known aspartame literature.

Aspartame metabolism

Aspartame or its metabolic products may have direct effects in the brain if they penetrate the brain and achieve the necessary level and distribution. Nutrition-induced changes of levels of compounds in the circulation could also have indirect effects, such as influence on cerebral uptake (transport) of structurally related compounds. To examine such effects it is important to establish what products are formed from aspartame and what their concentration in the blood is over a period of time. To extrapolate the effects in humans from those found in animal studies, it is necessary to establish species differences in the formation and elimination of such aspartame-derived products.

It has been suggested that in addition to the aspartame-induced changes in the levels of the amino acids, another important factor is not so much the absolute level of any amino acid but the concentration ratios of the amino acids to each other, especially the phenylalanine to tyrosine ratio.[12] This suggestion was based on evidence that phenylalanine lowers catecholamine synthesis in the brain, whereas tyrosine stimulates it. This consideration is pertinent to studies of phenylketonuria because in PKU tyrosine remains low and only phenylalanine is elevated, whereas in most animal models of this disease both amino acids are elevated. Because phenylalanine hydroxylase activity is significantly higher in rodent liver than in human liver, more tyrosine is formed from aspartame-derived phenylalanine following aspartame consumption in rodents than is formed in humans after a comparable dose. It has been reported[12] that only at 60-fold higher doses of aspartame do rodents show a plasma

Address reprint requests to Dr. Abel Lajtha at the Center for Neurochemistry, The N.S. Kline Institute for Psychiatric Research, 140 Old Orangeburg Rd., Orangeburg, NY 10962 USA.
Received March 22, 1993; accepted December 17, 1993.
(J. Nutr. Biochem. 5:266–283, 1994.)

phenylalanine/tyrosine ratio close to that in man. A more recent paper,[13] in disagreement with the need for a 60-fold higher dose in rodents, disputes the measurements and suggests that an aspartame dose in rodents five times that in humans is closer to bioequivalence. A comparison of ratios in rats and mice over a wide range of doses with actual ratios in humans suggests that phenylalanine/large neutral amino acids (Phe/LNAA) plasma ratios in rodents are equivalent at two to six times the human dosage of aspartame.[14] Because a greater portion of phenylalanine is converted to tyrosine in rodents than in primates, the Phe/LNAA ratio will be very different from one species to another.

The assumption was that after a dose of aspartame, primarily phenylalanine and tyrosine levels are elevated in the brain, and that tyrosine protects against the phenylalanine effects. It seems reasonable to be more concerned about phenylalanine because, as will be discussed later, the uptake by the brain of aspartic acid is much slower than that of phenylalanine, and the metabolism of aspartate and methanol is much faster. Because of this difference in metabolism, in most cases, even at the highest doses of aspartame, only phenylalanine was found to be increased in the blood, and aspartic acid and methanol levels were usually not affected.

The effect of phenylalanine is of particular concern because phenylketonuria is characterized by high phenylalanine levels, which are prevented by low phenylalanine in the diet. However, it has not been shown that phenylalanine to tyrosine ratios determine the toxic effects of phenylalanine in phenylketonuria, or that tyrosine provides therapeutic benefit in phenylketonuria or hyperphenylalaninemia. One tentative report suggested that supplementation of unrestricted diet with large amounts of tyrosine improved performance in three of five young adult phenylketonuria patients,[15] but a recent report could not confirm these results.[16]

In studies of the metabolism of aspartame, in each of several species (mouse, rat, rabbit, dog, monkey, human) aspartame was found to be completely hydrolyzed to the same products–aspartate, phenylalanine, and methanol.[17] Aspartame was found to undergo complete first-pass hydrolysis in the gut with no intact aspartame found in blood.[18] Intestinal esterases split off methanol, and dipeptidases further split the aspartyl phenylalanine to its constituent amino acids. Absorbed methanol and aspartic acid are metabolized to CO_2 via the one-carbon metabolic pool and the tricarboxylic acid cycle, primarily in the liver. Phenylalanine metabolism is somewhat slower, partly giving rise to tyrosine.[17] A recent study found similar rapid aspartame metabolism in young pigs.[18] In humans, infants and children metabolize aspartame to a degree similar to that of adults.[19–21] The metabolic sequence is that hydrolysis to methanol occurs first, followed by hydrolysis of the dipeptide before it enters the portal circulation. The simultaneous ingestion of sucrose with aspartame in normal adult subjects does not have a significant effect on the metabolism of the aspartame.[22] Diet (carbohydrate or protein) may influence the blood levels of aspartate, methanol, or phenylalanine, the products of aspartame metabolism, but it does not influence aspartame metabolism. In a large sample (108 male and female volunteers), subjects received either placebo or aspartame three times a day for 24 weeks at 75 mg/kg per day, which had no effect on plasma amino acid levels tested after at least 12 hours of fasting. Nonfasting values were not measured. In this study, no effects were found in an additional battery of tests measuring cholesterol, hematology, clinical chemistry, methanol, lipids, formate, creatinine and Ca excretion, etc.[23] The time course of change in phenylalanine levels in rodents following aspartame administration has been determined.[14]

As mentioned before, it is important to use doses, rates, and routes of administration that are equivalent to those of human aspartame use under normal conditions. To find effects of excessive ingestion and to establish safe levels, consumption of doses higher than usual must be examined, but the meaning of results with unrealistically high doses is questionable. Almost any compound at a very large dose will produce effects (most of them adverse) if the level in the brain becomes very high, including most of the nutrients essential for brain function. Whether aspartame administration was by solution or by capsule made only a small difference in the metabolism,[24] but capsule administration may be essential in blind testing to make it difficult to distinguish the placebo. Not only the amount but also the mode and timing of administration are important. Appearance of the products is faster with use of solutions,[25] but capsule administration is also suitable. Capsule doses of up to 20 mg/kg can be used for aspartame effect studies if the lower rate of absorption (lower peak concentration but identical area under the plasma concentration curve) of phenylalanine are taken into account.[24] At larger doses absorption may differ because of incomplete dissolution of the aspartame from the capsules.[25] Clearly, administration of a single dose that amounts to the daily intake does not represent usual consumption in a realistic manner. In our studies (to be discussed later), we chose to administer aspartame to rats in the drinking water[26–28] rather than as a single bolus. This extends the daily consumption over a longer period (primarily during the 8 to 12 hr when the animals are most active), thus producing plasma levels of metabolites very different from those occurring after a single bolus administration.

In aqueous solution, aspartame, like other dipeptides, is cyclized to some extent to a diketopiperazine product. The dipeptide formed from aspartame can also give rise to such a product,[29,30] which is metabolized further; plasma levels are below detectable levels even if the diketopiperazine is added to aspartame.[29] The absorption of diketopiperazine is slow.

These studies demonstrate that aspartame is very rapidly and completely broken down in the digestive tract, that its products, methanol and aspartic acid, are metabolized further, and that the major metabolite appearing in tissues is phenylalanine. The important questions are what dose of aspartame or which method of administration could result in increases in phenylalanine in plasma and brain greater than those that occur under physiological conditions, and what would the consequences of such increases be.

Dietary and aspartame-induced amino acid changes

Variability of amino acid levels

Although the levels of amino acids in the cerebral free amino acid pool are similar in most species, many changes in the concentration and regional distribution occur in an amino

acid-specific manner under a variety of conditions. Such changes show some differences between species. Because the amino acid composition of the plasma depends greatly on the diet (protein-rich or protein-poor) and undergoes major postprandial changes, large fluctuations in plasma amino acid levels are a frequent occurrence. For example, normal ranges of fasting and postprandial plasma concentrations in humans are, respectively, 3.2 ± 1.5 and 8.3 ± 3.3 μmol/L for aspartate and 47.2 ± 5.2 and 71.4 ± 8.2 μmol/L for phenylalanine.[1] Thus, plasma and tissue levels vary daily depending on nutritional status. For reference, the respective average intake of aspartate and phenylalanine is approximately 316 and 200 mg/kg body weight in children (4 years old) and 80 and 52 mg per kg body weight in adults.[1]

Because amino acids within the brain are not homogeneously distributed, they cannot be considered to be in a single pool functionally or metabolically. In a recent set of studies we reinvestigated the regional distribution of amino acids in the brain by assaying the levels in 53 different rat brain areas.[31,34] Large concentration differences were found for most amino acids, with the ratio of the level in the area with highest concentration to the level in the area of the lowest concentration varying from 6:1 to 15:1. For phenylalanine this ratio was 9:1. There are numerous areas in which levels are severalfold higher than in others,[33] and a number in which phenylalanine is severalfold higher than its average concentration in brain. This would indicate that, at least in some areas, phenylalanine at levels severalfold higher than average does not induce pathological changes. We repeated these assays in about the same number of human brain areas, and the distribution again showed severalfold differences in levels between the various areas.[35] The pattern of distribution is different for the different amino acids, with one area high in some amino acids and low in others. It is not known how nutritional elevation of total brain content influences such distribution, that is, which areas are increased more than others. Nor is it known how this heterogeneity influences amino acid metabolism, for example, in neurotransmitter formation from amino acid precursors that are at levels below or above average. It is also not known whether an increase in phenylalanine in plasma results in such a change in the brain in this amino acid, or in amino acids competing with it for transport into brain, that parallels the physiological distribution; that is, whether the increase occurs primarily in structures with high levels or is independent of endogenous levels, and whether decreased uptake of an amino acid that is competitively inhibited by increased phenylalanine results in regional changes that parallel regional distribution.

There are several factors that influence cerebral amino acid levels. In our studies examining changes in brain that are due to aging from adult to old,[31-34] in a number of areas changes of 10 to 50% were found. The changes were variable for each amino acid and area, and some showed increases, others decreases.

Large changes in the composition of the cerebral amino acid pool occur during development, with similar patterns in the various species but with some differences. The time and the rate of change are different for the various amino acids, with the levels of some changing rapidly in fetal or perinatal development, and those of others changing gradually during growth.[36] The effect of these developmental changes in levels on metabolism, function, neurotransmitter production, etc. has not been established.

What these findings show is that the cerebral amino acid pool is neither homogeneously distributed nor constant over development: there can be severalfold differences, regional or developmental, under physiological circumstances.

Further studies have been made in considerable detail on the changes seen in pathological conditions such as ischemia, stroke, and seizures, or under drug-induced pharmacological conditions. While these effects are significant in quantitative and functional terms, this topic is outside the scope of the present review. It is clear that the brain can tolerate significant changes in amino acid level and distribution, and that very large differences in the concentration of any amino acid exist among the various structures. An interesting question is whether some structures are more sensitive than others to significant changes in the levels of some amino acids.

Aspartame consumption

In any meaningful study of aspartame safety in humans, it is necessary to know rates of human aspartame use under normal conditions. In an American study, aspartame consumption in all age groups at the 90th percentile level was reported to be between 1.6 and 2.3 mg/kg body weight per day. Higher values were reported in a Canadian study (among users only)–5 to 9 mg/kg per day. These two studies are not necessarily contradictory–the Canadian study reported consumption measured in a single day, while the American study reported the average daily consumption measured over a 14-day interval. Because consumption can change from day to day, the lower value represents averages over a longer interval. Intake of aspartate and phenylalanine from aspartame among heavy users (i.e., 90th percentile) are approximately 0.9 and 1.2 mg/kg per day, respectively.[37] Such consumption would amount to only a low percentage of the dietary daily intake of the amino acid components of aspartame. In individuals restricting sugar intake (diabetics or those on a reducing diet), intake was 8 to 11 mg/kg per day,[37] well below the 40 to 50 mg/kg per day deemed acceptable by regulatory authorities, including the World Health Organization. The highest projected daily use of aspartame was estimated to be 22 to 34 mg/kg.[38] Most studies indicate that human consumption is below 10 mg/kg per day at the 90th percentile level.[3,4] In a few individuals consuming excessive amounts the use could be higher, up to 20 to 30 mg/kg per day. It is not likely that many persons exceed the 50 mg/kg per day intake that the Food and Drug Administration (USA) judges acceptable. The richest source of aspartame is likely to be diet soft drinks. A 12-oz bottle of soft drink could contain 180 mg of aspartame, corresponding to about 3 mg/kg for an adult or 9 mg/kg for a 20-kg child. Administration of a single bolus dose that amounts to the daily intake or several times that does not represent usual consumption in a realistic manner.

Aspartame effects on amino acid levels

Aspartame administered by gavage to rats at a dose of 200 mg/kg increased plasma and brain phenylalanine and tyrosine almost 100%. Such a dose would be equivalent in

sweetness to about 6 pounds of sugar for an adult man. Concurrent glucose administration, causing an insulin-mediated decrease in large neutral amino acids that compete with phenylalanine for transport from the circulation, further increased brain phenylalanine levels.[39] At these doses, brain tryptophan levels were occasionally decreased, but the levels of branched-chain amino acids in the brain were not affected.[40] After a single oral dose of aspartame to rats (250 or 1,000 mg/kg), maximal plasma phenylalanine and tyrosine levels were reached in 1 hr, decreasing by half in 2 hr.[41] Brain levels changed similarly and returned to control levels after 4 hr. In another set of studies in which the diet of the rats contained 5% aspartame with and without sucrose or protein, increases in phenylalanine and tyrosine were noted in plasma and brain after administration for 2 hr, and much smaller changes after a longer time on the diet (3 weeks).[42] In similar experiments using commercial chow, no significant changes in plasma or brain amino acids were observed with aspartame.[43] The dependence of plasma phenylalanine levels on the dose and the method of administration of aspartame has been reviewed.[14,38]

Plasma amino acid levels after aspartame administration to human subjects were measured by Stegink and coworkers in a series of papers.[44–47] When aspartame was administered in a single dose of 34 mg/kg, fasting plasma phenylalanine levels increased about 100% in 1 hr, tyrosine levels about 20%, returning to control in about 3 to 4 hr. Aspartate levels were not changed.[44] The experimental doses in these studies, such as 250 to 1000 mg/kg, even 34 mg/kg in a single dose, were higher than those of usual human consumption. Aspartame (34 mg/kg) in combination with monosodium glutamate did not increase plasma glutamate levels further.[45] Repeated aspartame administration (three doses of 10 mg/kg at 2-hr intervals) did not increase plasma aspartate levels but increased phenylalanine levels about 30%–returning to control levels each time between administrations–and resulted in increased Phe/LNAA ratios. These ratios were decreased when a protein meal was consumed with the aspartame.[46] When aspartame was administered at eight 1-hour intervals (in a dose equivalent to 9 liters of diet drink), plasma phenylalanine reached steady-state levels after four doses and did not exceed usual postprandial levels; aspartate levels were not increased.[47] When doses of aspartame mimicking usual consumption were given to fasted normal human volunteers (0.8 and 8 mg/kg alone or with carbohydrate), plasma amino acids were not altered.[48] In a recent study, a single dose of 1 g of aspartame, equivalent to 2 liters of diet drink, caused an increase in plasma phenylalanine levels of about 50%, similar to the increase when albumin alone was administered, but values returned to control more slowly than after an albumin meal when the aspartame was given; aspartate levels were not increased by aspartame; only by albumin.[49] When aspartame was given to rats at 200 mg/kg, brain tryptophan levels were occasionally reduced, but they were not affected by 50 or 100 mg/kg doses. An 8.3 mg/kg dose of aspartame as part of either a low or a high carbohydrate meal had no significant effect on plasma amino acid concentrations.[48] The half-life of phenylalanine in human blood plasma after various aspartame doses (34 to 200 mg/kg) was 1.7 hr.[38]

Stegink and coworkers in another set of studies examined the effect of aspartame administration on plasma amino acid levels in subjects with impaired phenylalanine metabolism—homozygous and heterozygous phenylketonurics.[50–54] In female obligate heterozygotes a dose of aspartame of 34 mg/kg increased plasma phenylalanine levels threefold, compared with twofold increases in normal controls; aspartic acid concentrations were not altered.[50] At lower concentrations (10 mg/kg) aspartame increased plasma phenylalanine 30% in controls and in heterozygotes, but aspartic acid levels were not affected.[51] To examine the effects of repeated aspartame ingestion, in one study doses of 10 mg/kg three times or 30 mg/kg once,[52] in another 10 mg/kg every 8 hours,[54] were given. The repeated doses in heterozygotes increased plasma phenylalanine about twofold, and the single dose about threefold. Note that these increased phenylalanine levels are within the postprandial range and are far below those reported to be neurotoxic during development in phenylketonuria. Caballero et al.[55] reported that baseline Phe/LNAA ratios in clinically normal mild hyperphenylalaninemics who do not require phenylalanine-restricted diets are about 0.85, a ratio considerably higher than that possible after aspartame consumption by normals or phenylketonuria-heterozygotes.

Recently, ratios of phenylalanine to large neutral amino acids in plasma were examined in mice and rats over a wide range of aspartame doses (up to 2 g/kg). Comparison of these data with those for humans indicated that rodents require two to six times higher doses of aspartame to produce the same Phe/LNAA ratios. In these studies, maximal plasma phenylalanine levels were reached within 1 hr of administration, and values returned to baseline in 1 to 4 hr depending on the dose.[41]

Phe/LNAA ratios in the heterozygous subjects were increased by aspartame from 0.13:1 to 0.24:1. This compares to a ratio of 0.37:1 in phenylketonuric children treated with a low phenylalanine diet, and of 3.5:1 for untreated individuals with phenyketonuria.[52] When subjects homozygous for phenylketonuria were given a single dose of 200 mg of aspartame (equivalent to a 12-oz drink) plasma amino acid levels were not altered. The change in plasma amino acids in response to carbohydrate was greater in phenylketonurics than in controls.[53] When 10 mg of aspartame per kg was given to diabetic patients with renal failure who were on maintenance hemodialysis, there was an increase in plasma phenylalanine and tyrosine. These increases were similar to postprandial increases.[56] A recent review came to the conclusion that feeding aspartame as a bolus dose of 34 mg/kg, representing a 99 percentile level of projected daily intake, repeated at 2-hr intervals, to normal adults, phenylketonuric heterozygotes, 1-year-olds, or insulin-dependent or non–insulin-dependent diabetics does not increase plasma phenylalanine levels above those experienced after ingesting a protein-containing meal.[57] In diabetic subjects with renal failure, aspartame at 10 mg/kg (corresponding to 25 packets of Equal, manufactured by The NutraSweet Co. of Deerfield, IL) caused increases only in plasma phenylalanine and tyrosine, which were in the postprandial range.[56] In these studies plasma amino acids were not significantly affected in humans or in experimental animals when aspartame doses comparable to normal consumption were given. Higher aspartame doses do elevate plasma phenylalanine levels

within the postprandial range; still higher doses are likely to increase cerebral phenylalanine levels as well.

The rationale for examining the Phe/LNAA ratio is that phenylalanine may inhibit the transport of other amino acids into the brain because of competition for shared transport sites. Phenylalanine is taken up by the large neutral amino acid (L) transport system. It has to be emphasized that the possible effects of phenylalanine are more complex, and transport may not be the only important aspect to study. In addition, the Phe/LNAA ratio may not always be a good predictor of changes in distribution.

The changes in substrates of the L amino acid transport system as a result of increased plasma phenylalanine levels are complex. This system can serve in the plasma-to-tissue and the tissue-to-plasma directions. Increased plasma phenylalanine may inhibit the transport of other large neutral amino acids into the brain (thus increasing their level in the blood); at the same time, increased levels of phenylalanine in tissues such as liver or brain would inhibit the transport of large neutral amino acids from the liver and brain (decreasing their blood level).[58,59] The transmembrane effects (heteroexchange) of intracellularly increased phenylalanine (phenylalanine exit stimulating the uptake of another amino acid) would also have to be taken into consideration. Thus, high plasma phenylalanine may inhibit tryptophan uptake into the brain, and high brain phenylalanine may inhibit tryptophan exit from the brain, and via heteroexchange phenylalanine exit may stimulate tryptophan uptake into brain. The sum of all these effects could be an actual increase in brain tryptophan, showing that plasma phenylalanine increase may not be a good predictor of cerebral tryptophan decrease. Further complexity was indicated by a study in which the decrease in brain tryptophan by high blood phenylalanine was prevented by adding lysine to blood.[60] Because the addition of lysine is unlikely to influence the inhibition of tryptophan uptake by phenylalanine, it suggests that a more complex mechanism is involved. In a study measuring amino acid uptake in human volunteers by positron emission tomography,[61] 34 mg/kg aspartame was administered in a single dose (corresponding to 5 liters of soft drink), which resulted in an 11.5% decrease in the transport rate constant. The conclusion was that little measurable transport change occurs under average (50th percentile) dietary use.

The available studies indicate that doses of aspartame that are equivalent to use at the 90 to 98 percentile level have little effect on plasma amino acids, but single large doses increase phenylalanine twofold to threefold and also increase tyrosine levels. One question that cannot be answered is what effect large doses have on the distribution of amino acids in human brain. It has been shown that phenylalanine shares the transport system that serves for its brain uptake with other large neutral amino acids.[62] Complex effects on transport processes seem to have an important role in phenylketonurias, apparently preventing depletion of cerebral amino acids in spite of the greatly elevated plasma phenylalanine, well above levels that would saturate the transport system. In any evaluation of amino acid level changes in the plasma it has to be taken into account that because fluctuation of amino acid levels in the plasma after a protein-containing meal occurs frequently, the brain is often exposed to such fluctuations under physiological conditions. These fluctuations involve most amino acids and result in altered concentration ratios of amino acids. These considerations and the lack of major changes in cerebral amino acids in phenylketonuria are indications that plasma phenylalanine fluctuations are unlikely to have important central effects.

Possible sites of neurochemical effects of aspartame

Amino acid metabolism

An increase in phenylalanine may also result in altered amino acid metabolism. Phenylalanine is metabolized in the liver via phenylalanine hydroxylase to tyrosine. An increase in tyrosine does not always parallel the increase in phenylalanine because at very high levels of phenylalanine the enzyme is saturated, and no further increase in tyrosine formation occurs. Increased phenylalanine in the retina was found to have no effect on the retinal tyrosine hydroxylation rate in rats if the hepatic phenylalanine metabolism was inhibited to mimic the human aspartame consumption model,[63] indicating that in humans even excessive aspartame doses are not likely to alter catecholamine formation. In the brain, these amino acids may alter tryptophan hydroxylation and thus serotonin synthesis, which has been shown to be influenced by brain tryptophan levels.[64,65] Similarly, elevated tyrosine levels in the brain can lead to increased norepinephrine synthesis.[66] Changes in amino acids in plasma can lead to changes in brain tryptophan and changes in brain serotonin content.[67] The effects of phenylalanine on catecholamine synthesis and release[68] were reported to be counteracted by tyrosine,[5,69] but the interactions of these two amino acids have not been clearly established. Of the aspartame doses that influence brain tryptophan levels and thus hydroxylation rates, only those of 530 mg/kg or higher had any effect beyond that observed after a protein meal;[70] this amount of aspartame is well beyond that ingested by human subjects as a single dose, even if a correction is made for species differences. The results indicate that after human aspartame consumption no significant alteration of the brain serotonin synthesis rate is likely to occur, even with large carbohydrate-induced increases in brain tryptophan levels.[70] Aspartame administration may result in an increase of alanine in the circulation, probably occurring because aspartame-derived aspartic acid metabolism gives rise to pyruvate, which is transaminated to alanine. In our studies in which aspartame was administered throughout pregnancy and lactation, we found a slight increase in plasma levels of alanine (17%) and phenylalanine (11%) in the offspring, with either no change or a slight, not significant, decrease in the brain levels of phenylalanine.[27]

Protein metabolism

The direct effect of aspartame administration on protein metabolism has not been examined; however, because plasma aspartate and phenylalanine levels remain within postprandial ranges even after excessive bolus doses, no effects are expected. One study testing the effect of 40 mg/kg aspartame in humans, dogs, and rats found no evidence of alteration of protein metabolism, but at the 4,000 mg/kg level in rats some effects of such high doses were found:

changes in liver cathepsin activity and plasma albumin half-life.[71]

The effect, not of aspartame, but of phenylalanine, on brain protein metabolism was examined in several studies, primarily in isolated systems. T.C. Johnson and co-workers published a series of papers on this subject in the 1970s, studying ribosomal systems.[72–75] In brain cell suspensions 14 mM phenylalanine decreased the concentration of intracellular amino acids and inhibited amino acid incorporation into proteins by 20%.[72] This concentration is severalfold higher than that reached in phenylketonuric brain. In neonatal mice a single injection of phenylalanine resulted in a decrease in polyribosomes and an increase in monoribosomes, and injection of large neutral amino acids after the phenylalanine caused reversal to control values.[73] Although phenylalanine reduced the level of intracellular amino acids, the level of RNA-bound amino acids was not decreased;[74] therefore, the effect on protein synthesis may not occur through a decrease in precursor levels. It is of interest that any single large neutral amino acid decreased polyribosome levels, whereas a mixture restored levels to normal. The initiator methionyl-tRNA levels were not restored–this could mediate the effects of phenylalanine.[75] It is not clear from these studies which proteins would be affected in these systems, or in general how long the effects would last, how great they would be, and how observations made primarily in isolated systems reflect effects under physiological conditions. The observed stimulatory influence of phenylalanine on ribonuclease[76] may indicate an effect on lysosomal degrading enzymes. The decrease in polyribosomes could be a postmortem effect, such as alteration by phenylalanine of ribonuclease activity in the homogenates during preparation of the ribosomes. Such a stimulation of ribonuclase activity may not occur in vivo.

Although phenylalanine may not affect overall protein synthesis, the synthesis or the breakdown of some specific protein fraction may be altered. Change in a small fraction may not be detectable when total brain proteins are examined. Effects of phenylalanine on incorporation into myelin protein have been reported.[77,78] The first study found inhibition of the transport of the labeled precursor amino acid, resulting in decreased incorporation of the label, with a somewhat greater decrease in myelin, which was interpreted to be a special effect on myelin protein synthesis.[77] In the second study, incorporation rates were too variable to interpret (glycine incorporation into myelin was increased), but the amount of myelin isolated was lower.[78]

In the early studies of amino acid effects on incorporation into proteins, the possible changes in the specific activity of the precursor that could influence measurements were not taken into account. We examined the effects of large flooding doses of amino acids when precursor specific activity was maintained, and no effect on incorporation was noted.[79] We came to the conclusion that the elevation of a single amino acid in brain (for example, of valine: several fold) does not affect the overall rate of cerebral protein synthesis. The problems in evaluating the results of the early experiments that used high concentrations of amino acids to study influences on cerebral amino acids were discussed in a review,[80] and additional work was suggested before any conclusion can be reached.

More recently, the effect of lower doses of phenylalanine was investigated in vivo using an internal carotid artery perfusion technique. In this study, a large increase in the phenylalanine concentration in the perfusate resulted in a decreased rate of leucine incorporation into brain proteins; at 864 μM phenylalanine the inhibition was 73%.[81] A concern in evaluating this study is that its control turnover values are considerably below the values usually reported. This could indicate some effect of the perfusion itself as used in the study, such as anoxia or heterogeneous precursor pools. It would be surprising to find a large inhibition of protein synthesis by elevated cerebral phenylalanine because elevation of other large neutral amino acids such as leucine and valine has no such effect.[79] Also, untreated homozygotes with chronic elevated phenylalanine levels below 1400 μM probably show no pathological changes.[82] To extrapolate the results of the study reporting inhibition of brain protein synthesis by the phenylalanine perfusion[81] to human conditions, the blood level of phenylalanine, which is always greatly elevated in phenylketonurics, would cause an almost complete inhibition of brain protein synthesis, a highly unlikely event in the presence of no observable behavioral or neurological changes in adult homozygotes previously maintained on a low phenylalanine diet. In our studies examining the effect of phenylalanine on protein synthesis by measuring the specific activity of the labeled free amino acid and that of the tRNA-bound amino acid and the rates of their incorporation into proteins,[83] we found no indication that when phenylalanine is elevated via infusion for the period of the experiment the rate of brain protein synthesis is altered. In early experiments it was shown that very high phenylalanine levels in the brain resulted in inhibition in polyribosomes of polypeptide chain elongation but when an amino acid mixture in addition to phenylalanine was administered, the system returned to normal in spite of similarly increased phenylalanine levels;[84] this was interpreted as being a large amino acid imbalance rather than phenylalanine toxicity resulting in inhibition of in vitro protein metabolism. In these early studies possible artifacts caused by changes in the labeled precursor pool were not examined.

The technical aspects of assaying cerebral protein metabolic rates are complex, and are not completely addressed in these studies. As already mentioned, the control metabolic rate in the perfusion study[81] was below the average rates reported in the literature, and the variations in the precursors were too large for accurate measurements. Most of the early experiments did not take into account the possibility of precursor compartments, or that the effects were on these compartments rather than on protein synthesis. Our results to date do not indicate a significant effect of phenylalanine on overall brain protein synthesis.

Effects on neurotransmitters

An acute dose (200 mg/kg) to rats was reported to result in increased blood and brain phenylalanine and tyrosine levels, but not in changes in brain dopamine, serotonin, or norepinephrine.[40,42] In another study in rats, oral administration of 200 or 250 mg of aspartame/kg with or without glucose or insulin failed to alter cerebral dopamine, norepinephrine, or serotonin levels.[43] In mice given aspartame orally at 13, 130,

and 650 mg/kg doses, respective increases of 12, 49, and 47% in norepinephrine in the hypothalamus were found after 3 hr.[85] In this study the reported norepinephrine increase in some brain areas was greater at the low dose than at the high dose. The changes in serotonin did not appear to be significant. A subsequent study from the same laboratory using these doses daily for 30 days found no change in norepinephrine or dopamine in the various brain regions, but at the high dose a decrease of serotonin was found in the hypothalamus.[86] A later study using a dose of 500 mg/kg and testing brain monoamine and metabolite levels after periods as long as 5 hr found no change.[41] Summarizing of these studies led to the conclusion that changes in cerebral neurotransmitter levels can be observed only after a single high dose of aspartame, which represents an artificial situation.[87,88] A study with rats given aspartame at 1,000 mg/kg orally found increases in brain phenylalanine and tyrosine, but no increase in monoamines, in the striatum, hippocampus, and nucleus accumbens.[89] Orally administered aspartame (1,000 mg/kg) in Fischer or Sprague-Dawley rats did not alter the regional levels of norepinephrine, dopamine, or serotonin measured in the 30- to 240-min period in seven brain areas.[90]

Although many of these reports found no effect even after unrealistically high aspartame doses, it has to be pointed out that nutritional influences on brain monoamine levels were reported in several instances. Brain tyrosine hydroxylase is not fully saturated under physiological conditions, and an increase in tyrosine levels after a protein-containing meal, for example, could lead to increased dopamine and norepinephrine levels and increased turnover of the amines. Administration of tyrosine resulted in an increase in dihydroxyphenylalanine (DOPA) synthesis. If phenylalanine was added, which also caused an increase in tyrosine levels, DOPA synthesis was not affected, leading to the suggestion that phenylalanine may inhibit the effects of tyrosine.[91] Increased neurotransmitter levels per se do not necessarily indicate changes in functional activity. Whether an experimentally induced increase in brain catecholamines represents an inactive extracellular accumulation followed by rapid metabolism of the exogenous catecholamines or represents increased catecholaminergic activity is not always clear. Although an increase in levels of the metabolites of neurotransmitter amines is often a sign of increased functional activity (increase in release followed by degradation), it is not always a reliable measure. It is possible that an increase in the precursor leads to the formation and subsequent degradation of a neurotransmitter in a pool that has no functional activity. In microdialysis experiments, dopamine levels were reported to be transiently elevated in the striatum of anesthetized rats following i.p. injection of 50 to 200 mg/kg of tyrosine. The authors hypothesized that increased dopamine release may have been due to increased synthesis caused by increased precursor availability, although the return of dopamine to baseline levels could not be explained by an accompanying decrease in brain tyrosine levels. These researchers reported that dopamine levels were transiently increased after low doses of phenylalanine and decreased after high doses of phenylalanine, the net effect being a rapid restabilization of dopamine to predosing levels.[92–94] Recent microdialysis experiments attempted to investigate whether precursor loading could lead to increased dopamine synthesis. These studies found, however, that an increase of tyrosine did not affect dopamine release either under normal conditions or when dopamine was increased. No coupling between dopamine synthesis and release was found.[92] An increase in tryptophan resulted in an increased level and release of serotonin.[95] Oral administration of 450 mg/kg of phenylalanine did not significantly change extracellular levels of dopamine or its metabolites as measured by microdialysis in baboon striatum.[96] In vivo release of dopamine measured by transstriatal dialysis was not changed after acute oral doses of 1,000 mg/kg of aspartame or 500 mg/kg of phenylalanine.[97] In one study, the effect of a large dose of glucose (3 g/kg) on brain serotonin levels was counteracted by a large dose of aspartame (200 g/kg).[39] A recent report did not find any effect of aspartame (250 mg/kg) on carbohydrate-induced tryptophan increase in the brain.[48]

To see whether use of aspartame for a longer time affects neurotransmitter systems, we measured receptor binding kinetics because binding may be up- or down-regulated when neurotransmitter levels are altered. In one set of studies we administered 50 and 500 mg/kg of aspartame per day, not as single doses but added to the drinking water for 30 days. With this method of administration, there were no significant changes in plasma and brain levels of phenylalanine, aspartic acid, or tyrosine, and no changes in levels in the brain (cortex, hippocampus, and striatum) of norepinephrine, dopamine, or serotonin.[26] The experimental animals were kept on a daily 12 hr light/12 hr dark cycle, and measurements were done during the middle of the dark cycle, when consumption was maximal. We also measured the binding kinetics of dopaminergic, adrenergic, and serotonergic receptors and found no changes in the level or affinity of receptors (K_d and B_{max}), assaying noradrenergic receptors with prazosin (α_1) and clonidine (α_2), serotonergic receptors with ketanserin (5-HT_2) and 80H dipropylaminotetraline (DPAT) (5-HT_{1A}), and dopaminergic receptors with SCH 23390 (D_1) and spiperone (D_2).[28] These results indicated that if aspartame is consumed gradually rather than as a single large dose, changes in amino acids are minimal and cerebral neurotransmitter systems are unaffected, with no change in neurotransmitter levels or in the level of binding affinity of the receptors, even if the daily consumption of aspartame is very high and is maintained over a period of time (1 month in our study).

Numerous studies have indicated that the developing brain is more sensitive to influences than the adult. This has also been shown in humans: homozygote phenylketonurics who were exposed to high phenylalanine levels during the early phases of their development show mental deficiency, whereas those not exposed during development but only during adulthood do not manifest the deficiency. Thus, above a threshold level during development phenylalanine shows toxicity, and the levels that are toxic for adults are much higher. Because of the greater sensitivity of the developing brain, one cannot make conclusions from experiments with adults on the possible effects in young, and it is important to test the effects of compounds on the developing brain. Few studies have examined effects of aspartame during the various stages of development.

Because of these considerations we repeated our experi-

ments of long-term aspartame exposure, examining possible aspartame effects on neurotransmitter systems in the developing brain. We administered aspartame at 500 mg/kg per day in the drinking water to rats throughout pregnancy and lactation and tested neurotransmitter systems in the brain of the 21-day-old weanlings. In the binding kinetics of adrenergic (α_2), serotonergic (5-HT_2), and dopaminergic (D_1 and D_2) receptors and in the levels of norepinephrine, serotonin, and dopamine, again we found no changes after this exposure to aspartame throughout development.[27] Other factors such as litter size or body weight were also unaffected. This large dose after exposure for a longer time did not have a measurable effect on the neurotransmitter systems examined.

One study examined the effect of aspartame in an isolated system rather than in the living animal. In these experiments aspartame was found to inhibit the binding of glutamate to its NMDA receptor, although this inhibition was weaker than that of aspartic acid.[98] This result indicates that the aspartic acid moiety of aspartame did retain some receptor affinity of the free aspartic acid in its peptide-bound form. While this is of interest, it does not have relevance to in vivo studies of aspartame effects. Because even after consumption of large doses, under most circumstances no intact aspartame can be found in the circulation, and because aspartame would not enter the brain even if plasma levels were significant, this observation does not give rise to concern for human use of aspartame. Changes in cerebral aspartic acid could influence NMDA receptor activity, but because aspartame does not increase aspartic acid levels in the brain, no change is expected. We have tested the possible effects of aspartame on NMDA receptors in weanling rats in experiments similar to those in which catecholaminergic receptors were examined.[27] These experiments indicated no change in excitatory amino acid receptors following aspartame exposure during development.[99] From the experiments discussed above we concluded that there is no evidence that aspartame use at fairly high levels, even for a prolonged period, would affect the neurotransmitter receptor systems in adults or in developing young. The stability of the brain is not surprising–one would expect functional stability of the neurotransmitter systems even if plasma constituents undergo major nutritional fluctuations.

Other metabolic effects

Many studies have examined nutritional aspects of aspartame, such as its effects on food consumption, appetite, food selection, hunger, thirst, etc. Although such nutritional effects may be influenced by central factors, this area is outside the scope of this review. Major changes in food intake, food selection, or water intake could affect metabolism in several ways. Recent articles examining the influence of aspartame on food intake in children[100] and adults[101] and on caloric intake,[102] hunger, and thirst,[103] do not indicate aspartame-induced changes that would have any effect on cerebral metabolism. It also must be emphasized that cerebral metabolism is better controlled and maintained than metabolism in the rest of the body, and even starvation or a low protein diet causes only minor changes in the brain compared with other organs.

One study examining the effects of 0.5 g of oral aspartame on serum hormone levels in humans found no change in prolactin, cortisol, growth hormone, or insulin in normal individuals.[104] Large doses of phenylalanine were reported to stimulate prolactin secretion. Clearly, in all effects of amino acids the experimental dose is of critical importance–very large doses may have various effects that are not observed under physiological conditions. This consideration is also of importance in evaluating reports that high phenylalanine levels affect tyrosine hydroxylation rates, because the effects of lower doses are unremarkable.[4] The assumption that carbohydrate diet-induced insulin release increases brain tryptophan and serotonin has been questioned, which would indicate that nutritional changes in plasma amino acids could, in feedback manner, alter carbohydrate appetite, and that aspartame could disrupt this feedback mechanism.[105]

In studies on possible pathological mechanisms responsible for mental deficiency in phenylketonuria, numerous reports have examined the effect of high levels of phenylalanine and of high levels of its metabolite phenylpyuric acid, which accumulates in phenylketonuria. Although at moderate levels little effect could be noted, at high levels phenylpyruvate especially was shown to inhibit some of the enzymes of glucose metabolism (hexokinase, pyruvate carboxylase, and citrate synthase) and ketone body and lipid metabolism.[80] Other phenylalanine metabolites were reported to have similar effects, but most of them could be observed only at levels in excess of those seen in phenylketonuric patients. It cannot be excluded that some brain structures, in young phenylketonurics, for example, contain much higher than average levels of phenylalanine or its metabolites and rather low levels of some of the enzymes, and thus inhibition of the enzyme might have a role in the disease. This does not seem to occur under normal conditions, however, or in adult phenylketonurics. It should be emphasized that although high phenylalanine levels are clearly causative in phenylketonuria, as shown by the prevention of pathological changes with a low phenylalanine diet, the mechanisms of the pathological changes are not known. Rather than phenylalanine itself, the pathology could involve one or more metabolites of phenylalanine that are produced in much greater amounts in phenylketonurics than in normal subjects given the same amount of phenylalanine. Thus, findings in phenylketonurics cannot be directly extrapolated to values for normal subjects.

Aspartic acid

Glutamic acid and aspartic acid, nonessential and rapidly metabolized amino acids, have an important and special function in the brain, because they may act (primarily glutamic acid) as excitatory neurotransmitters at glutamatergic receptor sites to which aspartic acid also shows affinity. Aspartame consists of equimolar amounts of phenylalanine and aspartic acid (the aspartic acid is 40% by weight), so the possible central effects of elevated aspartic acid also need to be considered. There are important differences between aspartic acid and phenylalanine. As already mentioned, aspartic acid metabolism is much faster, while its uptake into the brain is much slower. The level of aspartic acid in the brain is much higher than in the blood, and even a severalfold increase in blood does not result in a changed

brain concentration.[106] The exclusion by the blood-brain barrier of compounds that usually do not penetrate it, such as aspartic acid, is not absolute: when the level of a compound that is usually not taken up by the brain (such as taurine, glycine, glutamate) was increased in the blood many fold so that its blood level should be above its brain level, an increase could be seen.[107] The experimental conditions under which cerebral uptake could be shown are rather extreme and are not obtained under normal circumstances.

In examining the effects of 34 to 200 mg/kg aspartame in humans, no significant increase in plasma aspartic acid levels could be detected in adults or children.[47] When 1,000 mg/kg was given, plasma aspartic acid levels doubled in about 10 min and returned to control by 90 min;[49] even at peak plasma concentrations, plasma aspartic acid levels would still be well below brain levels, so no increase in the brain would be expected. Addition of monosodium glutamate and aspartame (34 mg/kg each) to a meal containing carbohydrates did not change plasma aspartate or glutamate levels from those seen after the meal alone. In the absence of carbohydrates the addition of monosodium glutamate to a meal increased plasma aspartate levels; the addition of aspartame caused a further small increase, with a maximal value of 50 μM at 30 min, returning to baseline at 150 min.[45] This 50 μM level is far below the toxic range because plasma levels must exceed 1,000 μM in young mice for neuronal damage to be observed.[3] Such neuronal damage is not noted in adults. Glutamic acid and related compounds could cause excitotoxic damage if taken up into brain tissue or into regions that are not protected by the blood-brain barrier. Reports of such excitotoxic neuronal damage by glutamic acid administration in neonatal monkeys are controversial at the present time, with one study reporting damage, and others reporting no effect.[8] As with phenylalanine and many other compounds, susceptibility of the nervous system to aspartic acid decreases with development in rodents, with threshold doses varying–220 mg/kg in newborn, 910 in weanling, and 1,900 in young (35-day-old) rats.[108] The 900 mg/kg aspartic acid threshold in weanlings is equivalent to a dose of 2,300 mg/kg of aspartame, a dose with the sweetness of 10 kg of sugar for a human child. Studies with aspartic acid demonstrate the importance of the dose used–at 750 or 1,000 mg/kg in young mice neuronal lesions were found in 100% of the experimental animals; at 650 mg/kg lesions were found in 30%; but at 500 mg/kg no lesions were found.[19] Such threshold values, which may be different in different species, emphasize the need to test compounds at realistic dose levels. Other factors, such as ingestion of food with aspartic acid, also have to be considered. The placental barrier to aspartic acid is strong, and an increase in the maternal circulation is not reflected in the fetal blood.[109] The blood-brain barrier, as already mentioned, also strongly excludes aspartic acid.[106] The threshold levels of aspartate for causing neuronal damage in young rodents and the protective effects of carbohydrates have been investigated in a number of laboratories, and there are some differences; however, no effects are recorded, at least at under 500 mg/kg aspartic acid or similar aspartame doses.[19,44]

In conclusion, although at very high doses of aspartame, especially together with monosodium glutamate, plasma aspartate levels do increase, aspartic acid levels in the brain under most conditions are not altered. As for excitatory amino acid levels and activities in brain and any possible excitotoxic effects, aspartame use even at very high levels does not appear to present the danger of adverse effects in human consumption.

Methanol

Aspartame, the methyl ester of aspartyl-phenylalanine, is approximately 10% methanol by weight. As discussed above, aspartame is hydrolyzed in the intestinal lumen to its component amino acids and methanol. Alternatively, some aspartame may be partially hydrolyzed to the aspartyl-phenylalanine dipeptide and methanol.[18] Peptide transport mechanisms may absorb aspartyl-phenylalanine directly into mucosal cells where proteolytic hydrolysis completes the breakdown to aspartate and phenylalanine.[110]

The methanol derived from aspartame has been cited as being of possible concern for neurotoxicity,[111] even though methanol exposure is much greater from other food stuffs such as fruits and juices.[112] Although it was formerly thought that formaldehyde formation was responsible for methanol toxicity, recent studies indicate that formic acid is the toxic agent, and that the toxic mechanism involves metabolic acidosis (with sodium bicarbonate protective at low methanol levels). Clinical studies indicate that a blood level of 1 g/L causes irreversible effects and that neurotoxic effects of methanol on the retina are a consequence of formate accumulation.[113-115] Aspartame consumption does not raise blood formate levels in humans, either on a theoretical basis[116] or experimentally.[47,117]

Methanol may be cleared more rapidly from the blood after low level dietary exposure, such as through aspartame or fruit juices, than it is after methanol loading.[21,118] At massive doses the half-life in the blood is 30 hr, while at low doses (human volunteers ingesting 1 to 5 mL) the blood half-life is 3 hr.[118] The time course for appearance and disappearance of serum methanol after both average and abuse levels of aspartame has been described. The sensitivity of conventional analytical techniques is inadequate for detecting methanol formation in humans, including children and adolescents, after aspartame dosages up to 34 mg/kg, the 99 percentile of projected daily consumption.[21,47,117,119] Davoli et al.,[120] using an analytical procedure capable of detecting small changes in blood methanol, reported a mean increase over endogenous basal levels of 1 mg/L methanol after consumption of 6 to 9 mg/kg aspartame by human volunteers. This increase was within the range of individual variation and values returned to the baseline 2 hours after aspartame administration. Abuse doses of aspartame of 200 mg/kg to adults and 100 mg/kg to infants resulted in mean peak blood methanol levels of 26 mg/L and 10 mg/L, respectively.[21,117] Blood methanol concentrations greater than 200 to 1,000 mg/L are required for clinical neurotoxicity or for measurable formate formation.[118,121] Fruit juice contains about three times as much methanol as an aspartame-sweetened beverage.[117]

Aspartylphenylalanine diketopiperazine

When dissolved in water, peptides can form cyclized compounds. Such products are found in many protein-containing

foods such as cheese, in protein hydrolysates, and heat-treated substances such as roasted malts. The cyclized product of aspartame is aspartylphenylalanine diketopiperazine. The cyclization depends on pH and temperature, and in 4 to 5 months at pH 4 as much as 20% of the aspartame in a drink can be converted to the cyclic product. Such product formation may be general for proteins in water or in condiments.[30] The compound formed from aspartame was tested for toxicity in a number of studies (acute, chronic, carcinogenicity, teratology, etc.). It was also tested in pharmacological studies looking for changes in the gastrointestinal, cardiovascular, and nervous systems, and the Joint Expert Committee on Food Additives established a "no observable effect level" of 750 mg/kg per day and an "acceptable daily intake" of 7.5 mg/kg per day. The Food and Drug Administration's corresponding values are 3,000 and 30 mg/kg per day. The estimated maximal possible intake of this compound at the 90th percentile level of aspartame consumption is 0.6 mg/kg per day. The compound is not known to be metabolized in the brain, but it undergoes hydrolysis to aspartic acid and phenylalanine by microorganisms in the gut. About 50% of an oral dose in humans is so converted.

When 200 mg/kg aspartame containing 10% of the diketopiperazine product was administered to humans, no diketopiperazine could be detected in the blood; about 5% was excreted intact in the urine,[29] and the rest was probably metabolized. Because of its slow absorption and slow metabolism[122] diketopiperazine reaches the circulation slowly as the cyclic compound itself, but because of the blood-brain barrier little if any is expected to reach the brain.

β Aspartame and phenylalanine methyl ester

The β aspartame isomer is a second, minor conversion product formed in stored beverages sweetened with aspartame (α aspartame); it is found in smaller amounts than the diketopiperazine compound. Its formation is related to storage time, temperature, and pH of the beverage. Recently, β aspartylphenylalanine, the free acid of β aspartame, was identified in the urine of healthy subjects who had not consumed aspartame; other β aspartyl dipeptides have been found in human urine.[123–125] A broad range of pharmacological and toxicological tests on β aspartame in animals have been reported. It has been tested in rats and dogs at 250, 500, and 1,000 mg/kg per day, given in the diet for 26 weeks followed by a 4-week aspartame-free diet, with no adverse effects noted.[123] When β aspartame was given to pregnant rabbits at 250 or 500 mg/kg per day no teratogenicity was found; maternotoxicity was evident at 750 mg/kg/day. Such a dose to rats caused a small decrease in body weight of the offspring at weaning.[123] In humans it was estimated that about 8% of the ingested amount is absorbed. β Aspartame is not hydrolyzed rapidly to its constituent amino acids in the small intestine but by gut bacteria, with further metabolism of its amino acids in the gut.[126]

Hydrolysis of the amino bond of aspartame can result in formation of the aqueous decomposition products of aspartate and phenylalanine methyl ester (PME). PME is a relatively minor product of aspartame decomposition compared with the diketopiperazine and β aspartame.[18] PME is rapidly metabolized in the intestinal lumen and enterocyte to form phenylalanine and methanol.[18,127] Intact PME is not detected in portal blood of young pigs after PME dosing at 450 mg/kg.[18] Absorbed PME would be rapidly hydrolyzed to phenylalanine and methanol in whole blood.[18] Thus, these data indicate that exposure of the central nervous system to PME following aspartame consumption is unlikely.

Clinical considerations of aspartame consumption

Phenylketonuria

From the previous discussions it is clear that studies of the possible central effects of aspartame should focus primarily on possible phenylalanine effects, because the levels of other metabolites very rarely reach significance. For research, phenylalanine is unique among amino acids in that we can now observe a population of phenylketonuric patients who had been treated with a low phenylalanine diet during their development but in adulthood are no longer on a low phenylalanine diet. Little observable neurological or mental symptoms have been reported in such subjects in the literature, although they have very high levels of phenylalanine in their blood chronically, with ratios of Phe/LNAA in plasma also greatly increased and most likely brain phenylalanine levels, too. The approximate fasting values for plasma phenylalanine levels (μM) are 45 for normal subjects, 70 for phenylketonuric heterozygotes (carriers), and often more than 1,000 for homozygotes. Although when maternal blood levels are above 800 the IQ of the offspring is affected, at levels lower than 600 there does not seem to be such an effect.[82] Such studies again demonstrate the already discussed difference in the toxicity threshold between the immature and the mature brain.

Normal subjects and heterozygotes have been studied often, but only a few studies have examined the effect of aspartame in homozygotes. One study examined the effect of a dose of 10 mg/kg aspartame on blood phenylalanine levels: normal subjects increased from 45 to 58 μM, heterozygotes from 69 to 82; while hyperphenylalaninemics at 412 μM and untreated phenylketonurics at 1,370 μM did not show any significant increase.[55] Aspartame packages carry a warning label alerting phenylketonurics that the compound contains phenylalanine; those persons on a phenylalanine-restricted diet need to take this into consideration.

It has to be emphasized that the mechanisms responsible for the pathological changes in phenylketonuria are not established. Whether these changes are direct effects of phenylalanine or its metabolic products on enzymes, hormones, or metabolism, or are indirect effects, involving alteration of transport or composition, is unclear at present. Present indications are that high phenylalanine causes pathological changes only in developing nervous systems, affecting developmental processes rather than adult brain function. Chronic exposure to high phenylalanine seems to be tolerated without significant toxic effects in most cases.

Several studies have examined plasma levels in heterozygotes of phenylketonuria after aspartame consumption. At 34 mg/kg aspartame, aspartic acid levels did not change in controls or heterozygotes, but phenylalanine levels (fasting) increased from 48 to 90 μM in controls, and from 59 to 150 in heterozygotes.[50] In another study with 10 mg/kg

aspartame, aspartic acid was not changed, but phenylalanine increased from 90 to 120.[51] With repeated doses (eight servings at 1-hr intervals totaling 600 mg of aspartame), plasma phenylalanine levels reached steady-state levels after five servings, and were slightly higher than postprandial levels; aspartic acid levels were not changed.[54] Similar effects were found when lower aspartame doses were used.[52]

In one study, effects on the electroencephalograms of children with generalized absence epilepsy were reported.[128] In another study, aspartame at 15 or 45 mg/kg per day did not have any measurable effect in heterozygotes when the electroencephalogram, urinary organic acids, and neuropsychological tests were examined for changes in neurological function.[129]

It was pointed out that in phenylketonuria the high blood levels of phenylalanine affect not only the brain amino acid pool, but also the pool in other organs, with differences in how the varied transport systems in the various organs are influenced.[58] The high level of phenylalanine seen in homozygotes and the relatively high level in maternal plasma required for having an effect on the developing offspring indicate tolerance to rather high levels of phenylalanine.

Clearly, aspartame consumption should be carefully monitored or avoided by anyone who is (or should be) on a phenylalanine-restricted diet. This would include pregnant homozygotes, young phenylketonuric children, and adult phenylketonurics on a low phenylalanine diet. It is recommended that plasma levels in such children be kept below 480 μM and in pregnant phenylketonurics below 380 μM. In a child on low phenylalanine diet (20 mg/kg instead of the normal 200 mg/kg per day), aspartame could increase the daily phenylalanine intake.

Pregnancy

Phenylketonuria is clear evidence of the much greater sensitivity of the developing nervous system to environmental changes. For this reason, diet during pregnancy requires special consideration.

An extensive 10-year study analyzing blood samples from a very large number of phenylketonuric and hyperphenylalaninemic mothers during pregnancy indicated a 600 μM plasma phenylalanine threshold for effects on fetal brain (as subsequently estimated by IQ measurements of the children).[130] The American Study for Maternal PKU recommends that during the pregnancy of phenylketonuric homozygotes, plasma phenylalanine levels be kept below 360 μM.[131] Peak plasma levels in normal subjects[132] and in phenylketonuric heterozygotes[133] were below such recommended levels when a bolus of 200 mg/kg aspartame was given (corresponding to 70 soft drinks in one dose). With a dose corresponding to 1 liter of soft drink no change in plasma phenylalanine could be detected; also, a dose of 10 mg/kg given to healthy subjects, mild hyperphenylalaninemics, and untreated phenylketonuric homozygotes did not result in any change in plasma phenylalanine concentration or in Phe/LNAA ratios.[53,55,132-134]

Consumption by a pregnant woman of no more than about 0.85 g of protein/kg body weight per day, as recommended by the World Health Organization,[135] would correspond to about 2.5 g of phenylalanine–an amount that is approximately 30 times higher than the phenylalanine contained in aspartame consumed by pregnant females at the 90th percentile level.[3] Pharmacokinetic studies demonstrate that it is not possible for normal or phenylkenoturia-heterozygous individuals to consume enough aspartame to elevate phenylalanine levels or ratios significantly above those of the postprandial state.[53,55,131-134] Even in mild, clinically normal hyperphenylalaninemics and phenylketonuria-homozygous individuals, the largest doses of aspartame likely to be consumed without additional protein do not further elevate phenylalanine levels or ratios from already high baselines.[55] Thus, whatever risk exists from dietary phenylalanine, such as in hyperphenylalaninemic pregnancies, the normal diet itself would pose a risk to the developing fetus as great as or greater than additional phenylalanine derived from aspartame. The effect of a dietary load of phenylalanine on a homozygote fetus of a heterozygote mother has not been examined, but extremely large doses of aspartame administered to phenylketonuria-heterozygotes do not raise phenylalanine levels or ratios even close to thresholds associated with adverse effects on fetal brain.

Effect on seizures

Animal studies. Aspartame, at a bolus dose of 1,000 or 2,000 mg/kg given orally to mice weanlings, was reported to increase seizure susceptibility.[11,136] The reason for these very high doses was to achieve greater increases in phenylalanine than in tyrosine. If pentylenetetrazol was given 1 hr after aspartame, the percentage of animals having convulsions was higher than that of those receiving pentylenetetrazol alone. A seizure-promoting effect of such aspartame doses was also reported in fluorothyl-treated weanling mice and in mice given electroshock. Equimolar phenylalanine had effects similar to those of aspartame, valine blocked aspartame effects, and neither aspartic acid nor methanol had any effect. Aspartame at 500 mg/kg or lower had no effect; 1,000 mg/kg aspartame given orally by bolus to fasted rats increased seizures induced by metrazol, but not seizures caused by quinolinic acid or electroshock. Phenylalanine had a similar effect. When the same amount of aspartame was given in three divided doses over a 2-hr period to fasted or fed animals, there was no effect on seizures. The respective increases in brain phenylalanine and tyrosine were 146% and 192% after a single dose, and 103% and 211% when given in three doses.[137] A number of similar studies could not find any effect of aspartame even at high doses on several seizure models. Oral administration of acute doses (50 to 2,000 mg/kg) or subchronic doses (up to 863 mg/kg per day) of aspartame in genetically epilepsy-prone rats had no facilitatory effect.[138] A number of subsequent papers reported that a similar high single dose of aspartame failed to produce proconvulsant effects. A single dose of 1,000 mg/kg by gavage or over a 14-day period had no effect in rats on kindling induced by prepyriform cortex stimulation, by electroconvulsive shock, or by pentylenetetrazol. In developing animals aspartame also had no proconvulsant effects.[139] Oral 1,000 mg/kg aspartame in rats was shown not to affect theophylline-induced seizures.[140]

While the above studies[139,140] were done on rats, a subsequent work on mice[141] repeated previous studies in which

potentiation of seizures was found[11,136] using similar conditions (the same strain of mice and the same convulsants). In this repeat study, aspartame up to 2,500 mg/kg failed to influence pentylenetetrazol-induced seizures. It also had no effect on brain norepinephrine or dopamine levels, but there were modest reductions in serotonin levels.[141] This work also indicated that a modest reduction in cerebral serotonin levels does not have toxic effects. At very high aspartame doses, changes in the serotonin system can be obtained, but they do not affect seizure threshold. These studies used the same procedures and animal strains as the previous positive study,[136] but were unable to reproduce the results. When measuring after-discharge threshold or seizure strength in rats after amygdala- and hippocampus-kindled seizures (in areas of low seizure threshold and having an important role in human epilepsy), aspartame from 25 to 2,000 mg/kg administered by gavage had no effect on seizure susceptibility.[142]

Recently, one study examined aspartame effects on metrazol-induced convulsions in guinea pigs and in two strains of mice given up to 2,000 mg/kg aspartame,[143] and another study examined the same dose in two types of genetically epilepsy-prone rats;[138] these studies and others failed to find seizure facilitation in any of these species. Very large acute doses of aspartame produced significant changes in plasma and brain amino acids, but alteration in monoamine neurotransmitter systems was largely absent. Some increases in norepinephrine at high doses were seen.[138] In baboons, which are sensitive to light-induced seizures, 1,000 mg/kg aspartame had no effect on seizure susceptibility or severity, although phenylalanine concentrations and phenylalanine to tyrosine ratios were similar to or higher than those in phenylketonuric humans.[144]

To see possible toxic effects of perinatal aspartame exposure, rats during pregnancy were given up to 1,600 mg/kg of aspartame daily, and later the pups were given up to twice as much in the drinking water with no effect on reflex development, morphological development, or spatial memory.[145] A recent review of studies of aspartame effects on seizure susceptibility reports that at a 1,000 mg/kg dose some studies found no effects and others found proconvulsant effects, but that excessive bolus doses do not appear to be relevant to human use and represent methodological problems.[146] Other reviews also conclude that current evidence suggests that aspartame consumption does not provoke seizures.[6,147]

Human studies. In human studies aspartame administered in a single morning dose of 34 mg/kg for 2 weeks failed to provoke seizures in children with epilepsy.[148] At 50 mg/kg divided into three equal doses at 2-hr intervals no effect on seizure incidence was found in children or adults who had previously reported seizures caused by aspartame consumption.[149] Studies of this kind do not indicate any effect of aspartame in children with various seizure disorders, including absence seizures.[150] In one study, heterozygotes received 15 or 45 mg/kg aspartame daily for 12 weeks with no observable electroencephalogram or cognitive effects.[151] Large chronic doses of aspartame (15 or 45 mg/kg per day in capsules for 12 weeks) had no effect on the electroencephalogram of 48 phenylketonuric heterozygotes in a detailed clinical evaluation.[129] A recent study of 10 children with unrelated newly diagnosed absence seizures found that the duration of spike wave discharge was increased by aspartame.[128] In this study there was no actual determination of absence seizures or of the effect of sucrose in controls and no comparable untreated period of the experimental subjects. The great variability of the spike-wave discharges in such children[152,153] and the lack of additional proper controls make the results difficult to evaluate and subject to alternative interpretations.[6,154,155]

In summary of the animal studies, there were a few positive reports on very high doses in some species with fluorothyl, electroshock, and pentylenetetrazol;[11,136] but the majority of the studies, including the use of various seizure models such as electroshock,[137] kindling,[139,142] genetically seizure-prone rats,[138] photosensitive epileptic baboons,[144] drug-induced seizures by lidocaine,[156] quinolinic acid,[137] or theophylline;[140] and repetition of the metrazol studies,[141,143] indicated that even unrealistically high oral doses of aspartame do not have significant effects on seizures.

Behavior

Although many studies have measured various aspects of behavior following aspartame administration, this subject is beyond the scope of this review; however, studies that examine central effects will be mentioned here very briefly. A recent book on aspartame[1] discusses a number of studies with negative findings on behavior. Among these studies were those examining the effect of sucrose on behavior using aspartame as a control. Other studies reporting no effects examined cognitive function in normal subjects[157] and in children;[130] effects on appetite and feeding also received detailed attention. In phenylketonuric heterozygotes the mean power frequency of the electroencephalogram showed a trend toward change between 300 and 400 μM plasma phenylalanine levels, with differences in response to identical phenylalanine levels among different individuals. At 1 μM in plasma, phenylalanine slowed performance on neuropsychological tests; changes of higher integrative function were also found.[158] Other behavioral tests on rodents, primates, and humans, including cognitive, sensory, motor, and neurophysiological tests, are summarized[3] with the conclusion that except in homozygotes, aspartame as a phenylalanine source has little significant effect. Adding 5% aspartame to the food of rats for 3 weeks did not influence feeding pattern, meal size, or spontaneous motor activity.[42] Perinatal exposure of rat pups to aspartame by first giving it to mothers (up to 1,600 mg/kg per day), then directly to pups (up to 3,600 mg/kg per day) via the drinking water, did not affect reflex development, morphological development, or spatial memory.[145] In human studies, a single dose of 60 mg/kg aspartame to male volunteers did not alter auditory, vigilance, IQ, motor, mood, or sleepiness tests.[157] In other behavioral studies in humans, a single dose of aspartame (15 mg/kg) did not affect mood, cognitive function, reaction time, or memory in 21- to 36-year-old volunteers.[159] Phenylalanine and aspartame, up to 10 g, tested in 20- to 35-year-old males did not affect energy, macronutrient selection, hunger, mood, or arousal.[160] Aspartame at 50 mg/kg tested in pilots did not affect perceptual, motor, or spatial activities; working

memory; attentional performance; risk taking; processing flexibility; planning; or sequencing ability.[161] In 9- to 10-year-old children, aspartame at 34 mg/kg did not affect learning, arithmetic calculation, activity level, social interaction, or mood.[162] In 30 preschool boys, including 12 reported to be sugar reactive with disruptive behavior, 30 mg/kg aspartame had no effect on aggression.[163] Thus, there seems to be no effect on behavior following high doses. Whereas one cannot conclude that aspartame ingestion interferes with cognitive functioning, the issue requires careful research under rigorous conditions.[164]

Conclusions

Aspartame administration in realistic amounts and manner

Because nutrient levels in the blood undergo large diurnal variations and depend on the daily diet, brain exposed to such a changing environment seems to be protected from damage. Although strong homeostatic and defensive mechanisms undoubtedly exist, it is important to examine possible acute and chronic effects of food additives on the central nervous system because even quantitatively small effects may be of importance.

After ingestion, aspartame is rapidly hydrolyzed to its three constituents methanol, aspartic acid, and phenylalanine, and very little or no intact compound reaches the circulation even after massive doses. The increase in methanol and aspartic acid in most studies is negligible, but even after excessive doses it is less than that occurring during regular nutritional cycles. Stored as a solution, aspartame is partially cyclized to a diketopiperazine product. The amount found will depend on the length of storage and the temperature during storage. This product is slowly hydrolyzed[122] and is excreted as such. Its level in the circulation is low under most conditions, and it does not seem to penetrate easily because it has not been detected in brain. Because of the low blood levels of aspartic acid, methanol, and the cyclized derivative, the major focus has been on the changes in blood phenylalanine levels.

Phenylketonuria

The questions to be asked are whether significant amino acid changes occur in persons consuming aspartame; whether such changes differ from those occurring under physiological conditions, such as those following a meal; and what the consequences of such increases are in brain function. In recent years, a new population has become available for study–phenylketonuric homozygotes, who during development were given a low phenylalanine diet to avoid pathological changes, and who as adults on normal diets exhibit greatly elevated phenylalanine levels.

Studies on phenylketonurics showed clearly that the developing nervous system is more sensitive than the adult to high phenylalanine levels. If levels in mothers exceed 600 μM, physical, pathological, or IQ changes in the offspring can usually be observed. The 600 μM blood level in the mother seems to be a threshold for damage to the fetus,[82] although the best outcome is, at least initially, blood phenylalanine levels in the child below 200 to 400 μM.[165] The important question for homozygotes is until what age and at what level is phenylalanine harmful to the nervous system. It is clear that the outcome is best for homozygotes if the mother is put on the low phenylalanine diet as early as possible, and the child is maintained on such a diet in spite of possible difficulties (economic, social, and biological) of such prolonged maintenance. It is not clear how long such a diet should be maintained, and what the explanations for the observed differences within the population are. Phenylketonuria may not be a homogeneous disease with homogeneous deficiencies, since the apoenzyme or the generation of cofactor may be affected, in different ways, which indicates the need to follow individuals separately and for a long time.[166] We are not aware of studies in which homozygotes were on a low phenylalanine diet till adolescence then followed till late adulthood, and those staying on the diet were compared with those taken off the diet, or those taken off were compared with controls. A number of studies indicate that at least some children are harmed if the low phenylalanine diet is discontinued early (before 10[167,168] or 12[169,170] years of age), with levels kept even lower under 6.[165,169,171] Because a few adults were tested before and after the low phenylalanine diet, and their pathology before the test is indefinite, it is not clear how long the diet should continue or whether resumption of the diet is beneficial, or whether prolonged diet may have side effects. Several suggestions have been made that the diet should be continued to some degree[172] and perhaps should be lifelong.[171] One could assume that any major pathological changes occurring in adult homozygotes (most of whom are not on a diet) would have been described, but a more careful and refined analysis of possible subtle neurological, intellectual, or emotional changes would be required to establish whether any effects can be identified.

The levels of plasma phenylalanine in humans given various doses of aspartame have been measured.[1–7] Changes in special populations have also been reviewed.[51–57] The general consensus is that at average use (the consumption of 90 to 99% of users) the changes in blood levels are below the range of those occurring after a protein-containing meal. In phenylketonuric homozygotes, changes and use need to be considered more carefully, and any changes in plasma phenylalanine levels should be carefully monitored (and avoided) during pregnancy or in those with phenylalanine levels already very high. Treatment of homozygote phenylketonurics with a diet low in phenylalanine began about 40 years ago. Nevertheless, there is no study available comparing adult homozygotes on such a diet with those not on it, hence no official dietary recommendation has been arrived at yet. The occurrence of phenylketonuria (homozygotes) is not rare–about one per 12,000 of the population. Although it is likely that major pathological changes would have been reported in this population, it is puzzling that detailed examinations have not been performed. The physiological levels of phenylalanine vary between 50 and 200 μM in the population; the phenylketonuria diet initially recommended would keep levels between 200 and 400 μM[165,167] for up to 8 years, and at 500 to 1,200 μM after that,[172] but a consensus based on available data on the desirable levels and the length of time they

needed to be maintained has not been reached. The use of more refined biological and behavioral tests and the separate examination of various patient groups depending on their defect (enzyme or coenzyme, etc.), previous history (when diet was started and for how long), family history, etc. is needed before safe and toxic phenylalanine thresholds can safely be established.

Animal models

It is not surprising that at high doses alterations can be effected: at excessively high doses, most normally harmless compounds can have ill effects. It is important, therefore, to know the appropriate doses and routes of administration to be examined. In considering the route, oral administration seems the most reasonable; in considering the dose, repeated realistic amounts more closely resembling actual use seem reasonable. There is disagreement on how closely values of animal models approach human values. It was proposed that because tyrosine may counteract phenylalanine effects, not the absolute level of phenylalanine, but the ratio of phenylalanine to tyrosine, is of importance; that is, phenylalanine should be in excess. The use of such a ratio assumes that phenylalanine is the toxic substance, and that tyrosine counteracts the toxic effects. This has not been demonstrated in a convincing manner. If the effect of phenylalanine is on large neutral amino acid transport, tyrosine effects are more likely to be additive rather than protective, for example. According to the ratio theory, high tyrosine administration would protect phenylketonurics against pathological changes–this has never been reported.

The consumption of aspartame, even among heavy users, is variable, and is not the same each day. At the 90th percentile level, human aspartame consumption, depending on age and body weight, is on average lower than 2 to 5 mg/kg per day and is rarely near the 35 mg/kg per day level[1–4,37]; with this level of intake, plasma amino acids remain in the normal range and are always well below the 50 to 200 mg/kg per day phenylalanine consumption of a normal diet. Most studies using realistic doses in humans and in primates in the proper ratio and using even larger doses in rodents, have not found phenylalanine levels that exceeded dietary variations.

Variations in cerebral levels

It is clear that brain components are not at constant levels but vary under nutritional, endocrine, developmental, aging influences, etc. Regional serotonin levels are changed after a meal, for example.[100] Regional studies have not been done with aspartame, but changes usually vary regionally and can occur even in opposite directions at different sites. Because changes in cerebral amino acids occur under physiological conditions, each change may not represent altered functional activity. Therefore, it is important to identify the specific process that has been altered and its functional consequences. A change of catecholamine levels may not indicate altered catecholaminergic activity. In the many reviews of aspartame activity[1–5] no toxic reactions were reported.

It is possible that the numerous animal and human studies have not uncovered some changes in minor components that are of significance–that trace amines or specific proteins are altered or transport at specific sites. However, it is worth mentioning again how nature has provided a chronically elevated level of phenylalanine in homozygous phenylketonurics. The studies on homozygous phenylketonurics show that human adults can tolerate chronic exposure to high phenylalanine remarkably well. At present there is no finding that would contraindicate the use of aspartame as a food additive.

Effects in humans

The available studies searching for possible physiological, nutritional, behavioral, cognitive, and pharmacological effects of aspartame use in normal subjects and in populations that could be sensitive–phenylketonurics, heterozygotes, children, including hyperactive children, people sensitive to seizures, diabetics, etc.–did not reliably establish any effect of normal aspartame use that would be deleterious. Because under normal aspartame use phenylalanine level changes are in the normal postprandial range, this is not unexpected.

Summary

1. Aspartame in its unchanged form does not attain significant blood levels even after massive doses. It is rapidly metabolized to its constituents aspartic acid, phenylalanine, and methanol. A minor cyclized diketopiperazine derivative is formed at low levels in some foods. Other than phenylalanine, the metabolic products are very rapidly metabolized further, and thus the compound of primary interest is phenylalanine.

2. Animal models are of use in aspartame studies, but the use of realistic doses and routes and intervals of administration is necessary for evaluating any possible effects. Under such experimental conditions there is no evidence that significant changes in brain metabolism, neurotransmitters, or receptor systems occur. Unrealistically high megadoses of most compounds could have undesirable effects, but these are not relevant to even the extremes of normal use.

3. In measuring behavior, performance, and plasma constituents of human subjects (adults and children) including sensitive populations (such as phenylketonurics kept below harmful threshold levels but much above physiological levels), there are no indications that aspartame use would be harmful, inducing seizures, hyperactivity, or decreased cognition or performance.

4. The negative findings of the numerous studies of the effects of aspartame administration under many different conditions and dose levels are to be expected because under most conditions the increase in plasma phenylalanine after heavy aspartame consumption is in the range of that occurring after consumption of a protein-containing meal. A further indication that high phenylalanine may not be neurotoxic is the lack at present of convincing evidence for pathological changes in the phenylketonurics who were treated during development with low phenylalanine diets and are on normal diets with high plasma phenylalanine levels as adults. There are no reports that even chronic exposure to high phenylalanine causes neuropathological changes in this population, especially if pathology was avoided by an early and prolonged low phenylalanine diet. Although further work may detect changes in homozygotes if their plasma

levels are not well controlled, it has to be emphasized that they represent a special, perhaps partially damaged, population with phenylalanine levels that are much higher than that achieved by any aspartame users.

5. The examination of aspartame represents a desirable example of a detailed and responsible study of possible effects of food additives on the nervous system. At present there is no indication of lack of safety in human consumption. These negative findings should not discourage future testing–all food additives should undergo such extensive, careful neurotoxic testing.

References

1. Stegink, L.D. and Filer, L.J., Jr. (1984). *Aspartame: Physiology and Biochemistry.* Marcel Dekker, New York, NY USA
2. Janssen, P.J.C.M. and van der Heijden, C.A. (1988). Aspartame: review of recent experimental and observational data. *Toxicology* **50,** 1–26
3. Butchko, H.H. and Kotsonis, F.N. (1989). Aspartame: review of recent research. *Comm. Toxicol.* 3, 253–278
4. Fernstrom, J.D. (1991). Central nervous system effects of aspartame. In *Sugars and Sweeteners,* (N. Kretchmer and C.B. Hallenbeck, eds.), p. 151–173, CRC Press, Boca Raton, FL USA
5. Fernstrom, J.D. (1984). Effects of acute aspartame ingestion on large neutral amino acids and monoamines in rat brain. In *Aspartame. Physiology and Biochemistry,* (L.D. Stegink and L.J. Filer Jr., eds.), p. 641–653, Marcel Dekker, New York, NY USA
6. Jobe, P.C. and Dailey, J.W. (1993). Aspartame and seizures. *Amino Acids* **4,** 197–235
7. Wurtman, R.J. and Ritter-Walker, E. (eds.) (1988). *Dietary Phenylalanine and Brain Function.* Birkhauser, Boston, MA USA
8. Olney, J.W. (1981). Excitatory neurotoxins as food additives: an evaluation of risk. *Neurotoxicology* **2,** 163–192
9. Maher, T.J. (1986). Neurotoxicology of food additives. *Neurotoxicology* **7,** 183–196
10. Pardridge, W.M. (1986). Potential effects of the dipeptide sweetener aspartame on the brain. *Nutr. Brain* **7,** 199–241
11. Maher, T.J. and Wurtman, R.J. (1987). Possible neurologic effects of aspartame, a widely used food additive. *Environ. Health Perspect.* **75,** 53–57
12. Wurtman, R.J. and Maher, T.J. (1988). Calculation of the aspartame dose for rodents that produces neurochemical effects comparable to those occurring in people. In *Dietary Phenylalanine and Brain Function,* (R.J. Wurtman and E. Ritter-Walker, eds.), p. 144–148, Birkhauser, Boston, MA USA
13. Fernstrom, J.D. (1989). Oral APM and plasma phenylalanine: pharmacokinetic difference between rodents and man, and relevance to CNS effects of phenylalanine. *J. Neural. Transm.* **75,** 159
14. Hjelle, J.J., Dudley, R.E., Marietta, M.P., Sanders, P.G., Dickie, B.C., Brisson, J., and Kotsonis, F.N. (1992). Plasma concentrations and pharmacokinetics of phenylalanine in rats and mice administered aspartame. *Pharmacology* **44,** 48–60
15. Lykkelund, C., Nielsen, J.B., Lou, H.C., Rasmussen, V., Gerdes, A.M., Christensen, E., and Guttler, F. (1988). Increased neurotransmitter biosynthesis in phenylketonuria induced by phenylalanine restriction or by supplementation of unrestricted diet with large amounts of tyrosine. *Eur. J. Pediatr.* **148,** 238–245
16. Pietz, J., Pietz, B., Kutscha, A., Schmidt, H., Tretz, F., and de Sonneville, L. (1993). Tyrosin-supplementierung als alternatives behandlungs-konzept fur patienten mit phenylketonurie (PKU)? Ergebnisse einer placebo-kontrollierten crossover-doppelblindstudie. *Monatschrift Kinderheilkunde* **141,** S43
17. Ranney, R.E., Oppermann, J.A., Muldoon, E., and McMahon, F.G. (1976). Comparative metabolism of aspartame in experimental animals and humans. *J. Toxicol. Environ. Health* **2,** 441–451
18. Burgert, S.L., Andersen, D.W., Stegink, L.D., Takeuchi, H., and Schedl, H.P. (1991). Metabolism of aspartame and its L-phenylalanine methyl ester decomposition product by the porcine gut. *Metabolism* **40,** 612–618
19. Filer, L.J., Jr., Baker, G.L., and Stegink, L.D. (1984). Aspartate ingestion by human infants. In *Aspartame,* (L.D. Stegink and L.J. Filer, Jr., eds.) Marcel p. 579–591, Dekker, New York, NY USA
20. Filer, L.J., Jr., Baker, G.L., and Stegink, L.D. (1983). Effect of aspartame loading on plasma and erythrocyte free amino acid concentrations in one-year-old infants. *J. Nutr.* **113,** 1591–1599
21. Stegink, L.D., Brummel, M.C., Filer, L.J., Jr., and Baker, G.L. (1983). Blood methanol concentrations in one-year-old infants administrated graded doses of aspartame. *J. Nutr.* **113,** 1600–1606
22. Stegink, L.D., Brummel, M.C., Persoon, T.J., Filer, L.J., Jr., Bell, E.F., and Ziegler, E.E. (1990). Effect of sucrose on the metabolic disposition of aspartame. *Am. J. Clin. Nutr.* **52,** 335–341
23. Leon, A.S., Hunninghake, D.B., Bell, C., Rassin, D.K., and Tephly, T.R. (1989). Safety of long-term large doses of aspartame. *Arch. Intern. Med.* **149,** 2318–2324
24. Burns, T.S., Stargel, W.W., and Hurwitz, A. (1990). Bioavailability of phenylalanine and aspartate from aspartame (20 mg/kg) in capsules and solution. *Metabolism* **39,** 1200–1203
25. Stegink, L.D., Filer, L.J., Jr., Bell, E.F., and Ziegler, E.E. (1987). Plasma amino acid concentrations in normal adults administered aspartame in capsules or solution: lack of bioequivalence. *Metabolism* **36,** 507–512
26. Reilly, M.A., Debler, E.A., Fleischer, A., and Lajtha, A. (1989). Chronic aspartame ingestion does not alter cerebral levels of aminergic neurotransmitters and related amino acids in the rat brain. *Res. Commun. Physiol. Psych. Behav.* **14,** 287–303
27. Reilly, M.A., Debler, E.A., and Lajtha, A. (1990). Perinatal exposure to aspartame does not alter aminergic neurotransmitter systems in weanling rat brain. *Res. Commun. Psychol. Psych. Behav.* **15,** 141–159
28. Reilly, M.A., Debler, E.A., Fleischer, A., and Lajtha, A. (1989). Lack of effect of chronic aspartame ingestion on aminergic receptors in rat brain. *Biochem. Pharmacol.* **38,** 4339–4341
29. Cho, E.S., Coon, J.D., and Stegink, L.D. (1987). Plasma and urine diketopiperazine concentrations in normal adults ingesting large quantities of aspartame. *Food Chem. Toxicol.* **25,** 499–504
30. Gaines, S.M. and Bada, J.L. (1988). Aspartame decomposition and epimerization in the diketopiperazine and dipeptide products as a function of pH and temperature. *J. Org. Chem.* **53,** 2757–2764
31. Banay-Schwartz, M., Lajtha, A., and Palkovits, M. (1989). Changes with aging in the levels of amino acids in rat CNS structural elements I. Glutamate and related amino acids. *Neurochem. Res.* **14,** 555–562
32. Banay-Schwartz, M., Lajtha, A., and Palkovits, M. (1989). Changes with aging in the levels of amino acids in rat CNS structural elements II. Taurine and small neutral amino acids. *Neurochem. Res.* **14,** 563–570
33. Banay-Schwartz, M., Lajtha, A., and Palkovits, M. (1990). Changes with aging in the levels of amino acids in rat CNS structural elements: III. Large neutral amino acids. *J. Neurosci. Res.* **26,** 209–216
34. Banay-Schwartz, M., Lajtha, A., and Palkovits, M. (1990). Changes with aging in the levels of amino acids in rat CNS structural elements: IV. Methionine and basic amino acids. *J. Neurosci. Res.* **26,** 217–223
35. Banay-Schwartz, M., Palkovits, M., and Lajtha, A. (1993). Heterogeneous distribution of functionally important amino acids in brain areas of adult and aging humans. *Neurochem. Res.* **18,** 417–423
36. Lajtha, A. and Toth, J. (1973). Perinatal changes in the free amino acid pool of the brain in mice. *Brain Res.* **55,** 238–241
37. Butchko, H.H. and Kotsonis, F.N. (1991). Acceptable daily intake vs actual intake: the aspartame example. *J. Am. Coll. Nutr.* **10,** 258–266
38. Filer, L.J., Jr. and Stegink, L.D. (1988). Effect of aspartame on plasma phenylalanine concentration in humans. In *Dietary Phenylalanine and Brain Function,* (R.J. Wurtman and E. Ritter-Walker, eds.), p. 18–40, Birkhauser, Boston, MA USA
39. Yokogoshi, H., Roberts, C.H., Caballero, B., and Wurtman, R.J. (1984). Effects of aspartame and glucose administration on brain and plasma levels of large neutral amino acids and brain 5-hydroxyindoles. *Am. J. Clin. Nutr.* **40,** 1–7
40. Fernstrom, J.D., Fernstrom, M.H., and Gillis, M.A. (1983). Acute effects of aspartame on large neutral amino acids and monoamines in rat brain. *Life Sci.* **32,** 1651–1658
41. Romano, M., Diomede, L., Guiso, G., Caccia, S., Perego, C., and

Salmona, M. (1990). Plasma and brain kinetics of large neutral amino acids and of striatum monoamines in rats given aspartame. *Food Chem. Toxicol.* **28,** 317–321

42. Torii, K., Mimura, T., Takasaki, Y., and Ichimura, M. (1986). Dietary aspartame with protein on plasma and brain amino acids, brain monoamines and behavior in rats. *Physiol. Behav.* **36,** 765–771
43. Torii, K., Mimura, T., Takasaki, Y., and Ichimura, M. (1986). Effect of mealing on plasma and brain amino acid, and brain monoamine in rats after oral aspartame. *Physiol. Behav.* **36,** 759–764
44. Stegink, L.D., Filer, L.J., Jr., and Baker, G.L. (1977). Effect of aspartame and aspartate loading upon plasma and erythrocyte free amino acid levels in normal adult volunteers. *J. Nutr.* **107,** 1837–1845
45. Stegink, L.D., Filer, L.J., Jr., and Baker, G.L. (1987). Plasma amino acid concentrations in normal adults ingesting aspartame and monosodium L-glutamate as part of a soup/beverage meal. *Metabolism* **36,** 1073–1079
46. Stegink, L.D., Filer, L.J., Jr., and Baker, G.L. (1988). Repeated ingestion of aspartame-sweetened beverage: effect on plasma amino acid concentrations in normal adults. *Metabolism* **37,** 246–251
47. Stegink, L.D., Filer, L.J., Jr., Bell, E.F., Ziegler, E.E., and Tephly, T.R. (1989). Effect of repeated ingestion of aspartame-sweetened beverage on plasma amino acid, blood methanol, and blood formate concentrations in normal adults. *Metabolism* **38,** 357–363
48. Romano, M., Casacci, F., De Marchi, F., Pacei, T., Esteve, A., Lomuscio, G., Mennini, T., and Salmona, M. (1989). Effects of aspartame and carbohydrate administration on human and rat plasma large neutral amino acid levels and rat brain amino acid and monoamine levels. *J. Nutr.* **119,** 75–81
49. Mellerm, S.F. (1991). Effect of aspartame and protein, administered in phenylalanine-equivalent doses, on plasma neutral amino acids, aspartate, insulin and glucose in man. *Pharmacol. Toxicol.* **68,** 408–412
50. Stegink, L.D., Koch, R., Blaskovics, M.E., Filer, L.J., Jr., Baker, G.L., and McDonnell, J.E. (1981). Plasma phenylalanine levels in phenylketonuric heterozygous and normal adults administered aspartame at 34 mg/kg body weight. *Toxicology* **20,** 81–90
51. Stegink, L.D., Wolf-Novak, L.C., Filer, L.J., Jr., Bell, E.F., Ziegler, E.E., Krause, W.L., and Brummel, M.C. (1987). Aspartame-sweetened beverage: effect on plasma amino acid concentrations in normal adults and adults heterozygous for phenylketonuria. *J. Nutr.* **117,** 1989–1995
52. Stegink, L.D., Filer, L.J., Jr., Baker, G.L., Bell, E.F., Ziegler, E.E., Brummel, M.C., and Krause, W.L. (1989). Repeated ingestion of aspartame-sweetened beverage: effect on plasma amino acid concentrations in individuals heterozygous for phenylketonuria. *Metabolism* **38,** 78–84
53. Wolf-Novak, L.C., Stegink, L.D., Brummel, M.C., Persoon, T.J., Filer, L.J., Jr., Bell, E.F., Ziegler, E.E., and Krause, W.L. (1990). Aspartame ingestion with and without carbohydrate in phenylketonuric and normal subjects: effect on plasma concentrations of amino acids, glucose and insulin. *Metabolism* **39,** 391–396
54. Stegink, L.D., Filer, L.J., Jr., Bell, E.F., Ziegler, E.E., Tephly, T.R., and Krause, W.L. (1990). Repeated ingestion of aspartame-sweetened beverages: further observations in individuals heterozygous for phenylketonuria. *Metabolism* **39,** 1076–1081
55. Caballero, B., Mahon, B.E., Rohr, F.J., Levy, H.L., and Wurtman, R.J. (1986). Plasma amino acid levels after single-dose aspartame consumption in phenylketonuria, mild hyperphenylalaninemia and heterozygous state for phenylketonuria. *J. Pediat.* **109,** 668–671
56. Gupta, V., Cochran, C., Parker, T.F., Long, D.L., Ashby, J., Gorman, M.A., and Liepa, G.U. (1989). Effect of aspartame on plasma amino acid profiles of diabetic patients with chronic renal failure. *Am. J. Clin. Nutr.* **49,** 1302–1306
57. Filer, L.J., Jr. and Stegink, L.D. (1989). Aspartame metabolism in normal adults, phenylketonuric heterozygotes, and diabetic subjects. *Diabetes Care* **12,** 67–74
58. Christensen, H.N. (1986). Where do the depleted plasma amino acids go in phenylketonuria? *Biochem. J.* **236,** 929–930
59. Christensen, H.N. and de Cespedes, C. (1988). Dual role of transport competition in amino acid deprivation of the central nervous system by hyperphenylalaninemia. In *Dietary Phenylalanine and Brain Function,* (R.J. Wurtman and E. Ritter-Walker, eds.) p. 68–78, Birkhauser, Boston, MA USA
60. Huether, G. (1986). The depletion of tryptophan and serotonin in the brain of developing hyperphenylalaninemic rats is abolished by the additional administration of lysine. *Neurochem. Res.* **11,** 1663–1668
61. Koeppe, R.A., Shulkin, B.L., Rosenspire, K.C., Shaw, L.A., Betz, A.L., Mangner, T., Price, J.C., and Agranoff, B.W. (1991). Effect of aspartame-derived phenylalanine on neutral amino acid uptake in human brain: a positron emission tomography study. *J. Neurochem.* **56,** 1526–1535
62. Oldendorf, W.H. and Szabo, J. (1976). Amino acid assignment to one of three blood-brain barrier amino acid carriers. *Am. J. Physiol.* **230,** 94–98
63. Fernstrom, J.D., Fernstrom, M.H., and Massoudi, M.S. (1991). In vivo tyrosine hydroxylation in rat retina: effect of aspartame ingestion in rats pretreated with p-chlorophenylalanine. *Am. J. Clin. Nutr.* **53,** 923–929
64. Lovenberg, W., Jequier, E., and Sjoerdsma, A. (1968). Tryptophan hydroxylation in mammalian systems. *Adv. Pharmacol.* **6A,** 21–36
65. Carlsson, A. and Lindqvist, M. (1978). Dependence of 5-HT and catecholamine synthesis on concentrations of precursor amino acids in rat brain. *Naunyn-Schmied. Arch. Pharmacol.* **303,** 157–164
66. Gibson, C.J. and Wurtman, R.J. (1978). Physiological control of brain norepinephrine synthesis by brain tyrosine concentration. *Life Sci.* **22,** 1399–1406
67. Fernstrom, J.D. and Wurtman, R.J. (1972). Brain-serotonin content: physiological regulation by plasma neutral amino acids. *Science* **178,** 414–416
68. Milner, J.D., Irie, K., and Wurtman, R.J. (1986). Effects of phenylalanine on the release of endogenous dopamine from rat striatal slices. *J. Neurochem.* **47,** 1444–1448
69. Milner, J.D. and Wurtman, R.J. (1985). Tyrosine availability determines stimulus-evoked dopamine release from rat striatal slices. *Neurosci. Lett.* **59,** 215–220
70. Fernstrom, J.D., Fernstrom, M.H., and Grubb, P. (1986). Effects of aspartame ingestion on the carbohydrate-induced rise in tryptophan hydroxylation rate in rat brain. *Am. J. Clin. Nutr.* **44,** 195–205
71. Tutelian, V.A., Gapparov, M.M., Virovets, O.A., Kravchenko, L.V., and Vasilev, A.V. (1989). New data on the metabolism and physiologic mechanisms of aspartame action. *Voprosy Pitaniia* **1,** 20–25
72. Hughes, J.V. and Johnson, T.C. (1975). The effects of phenylalanine on amino acid metabolism and protein synthesis in brain cells in vitro. *J. Neurochem.* **26,** 1105–1113
73. Hughes, J.V. and Johnson, T.C. (1977). Hyperphenylalanemia: effect on brain polyribosomes can be partially reversed by other amino acids. *Science* **195,** 402–204
74. Hughes, J.V. and Johnson T.C. (1977). The effects of hyperphenylalaninaemia on the concentrations of aminoacyl-transfer ribonucleic acid in vivo. *Biochem. J.* **162,** 527–537
75. Hughes, J.V. and Johnson, T.C. (1978). Experimentally induced and natural recovery from the effects of phenylalanine on brain protein synthesis. *Biochim. Biophys. Acta* **517,** 473–485
76. Roberts, S. (1974). Effects of amino acid imbalance on amino acid utilization, protein synthesis and polyribosome function in cerebral cortex. In *Aromatic Amino Acids in the Brain,* (Ciba Foundation Symposium 22), p. 299–324, Elsevier, Amsterdam, North-Holland
77. Agrawal, H.C., Bone, A.H., and Davison, A.N. (1970). Effect of phenylalanine on protein synthesis in the developing rat brain. *Biochem. J.* **117,** 325–331
78. Figlewicz, D.A. and Druse, M.J. (1980). Experimental hyperphenylalaninemia: effect on central nervous system myelin fractions. *Exp. Neurol.* 67, 315–329
79. Dunlop, D.S., van Elden, W., and Lajtha, A. (1975). A method for measuring brain protein synthesis rates in young and adults rats. *J. Neurochem.* **24,** 337–344
80. Roberts, S. (1985). Experimental aminoacidemias. In *Handbook of Neurochemistry,* vol. 9, (A. Lajtha, ed.), p. 203–218, Plenum Publishers, New York, NY USA
81. Wall, K.M. and Pardridge, W.M. (1990). Decreases in brain protein synthesis elicited by moderate increases in plasma phenylalanine. *Biochem. Biophys. Res. Commun.* **168,** 1177–1183
82. Levy, H.L. and Waisbren, S.E. (1983). Effects of untreated maternal phenylketonuria and hyperphenylalaninemia on the fetus. *N. Engl. J. Med.* **309,** 1269–1274
83. Dunlop, D.S., Yang, X., and Lajtha, A. (1993). Elevated plasma

phenylalanine and brain protein synthesis rates. *Proc. Am. Soc. Neurochem.* **24,** 113
84. Binek-Singer, P. and Johnson, T.C. (1982). The effects of chronic hyperphenylalaninaemia on mouse brain protein synthesis can be prevented by other amino acids. *Biochem. J.* **206,** 407–414
85. Coulombe, R.A., Jr. and Sharma, R.P. (1986). Neurobiochemical alterations induced by the artificial sweetener aspartame (NutraSweet). *Toxicol. Appl. Pharmacol.* **83,** 79–85
86. Sharma, R.P. and Coulombe, R.A., Jr. (1987). Effects of repeated doses of aspartame on serotonin and its metabolite in various regions of the mouse brain. *Food Chem. Toxicol.* **25,** 565–568
87. Fernstrom, J.D. (1983). The role of precursor availability in the control of monoamine biosynthesis in brain. *Physiol. Rev.* **63,** 484
88. Vhang, C., Bordet, S., Karoum, F., and Commissiong, J.W. (1990). Effect of precursors on the synthesis of catecholamines and on neurotransmission in the superior cervical ganglion of the rat. *J. Neuro. Chem.* **55,** 890–898
89. Perego, C., De Simoni, M.G., Fodritto, F., Raimondi, L., Diomede, L., Salmona, M., Algeri, S., and Garattini, S. (1988). Aspartame and the rat brain monoaminergic system. *Toxicol. Lett.* **44,** 331–339
90. Freeman, G., Sobotka, T., and Hattan, D. (1990). Acute effects of aspartame on concentrations of brain amines and their metabolites in selected brain regions of Fischer 344 and Sprague-Dawley rats. *Drug Chem. Toxicol.* **13,** 113–133
91. Wurtman, R.J., Larin, F., Mostafapour, S., and Fernstrom, J.D. (1974). Brain catechol synthesis: control by brain tyrosine concentration. *Science* **185,** 183–184
92. During, M.J., Acworth, I.N., and Wurtman, R.J. (1988). Effects of systemic L-tyrosine on dopamine release from rat corpus stritum and nucleus accumbens. *Brain Res.* **452,** 378–380
93. During, M.J., Acworth, I.N., and Wurtman, R.J. (1988). Phenylalanine administration influences dopamine release in the rat's corpus striatum. *Neurosci. Lett.* **93,** 91–95
94. During, M.J., Acworth, I.N., and Wurtman, R.J. (1989). Dopamine release in rat striatum: physiological coupling to tyrosine supply. *J. Neurochem.* **52,** 1449–1454
95. Westerink, B.H.C. and De Vries, J.B. (1991). Effect of precursor loading on the synthesis rate and release of dopamine and serotonin in the striatum: a microdialysis study in conscious rats. *J. Neurochem.* **56,** 228–233
96. Arvin, B., Chapman, A.G., and Meldrum, B.S. (1992). Extracellular levels of monoamines in striatum of baboon and rat following oral phenylalanine. *Soc. Neurosci. Abstr.* Vol. 18, p. 24
97. Garattini, S., Caccia, S., Romano, M., Diomede, L., Guiso, G., Vezzani, A., and Salmona, M. (1988). Studies on the susceptibility to convusions in animals receiving abuse doses of aspartame. In *Dietary Phenylalanine and Brain Function,* (P.J. Wurtman and E. J. Ritter-Walker, eds.), p. 130–143
98. Pan-Hou, H., Suda, Y., Ohe, Y., Sumi, M., and Yoshioka, M. (1990). Effect of aspartame on N-methyl-D-aspartate-sensitive L-[^{3}H]glutamate binding sites in rat brain synaptic membranes. *Brain Res.* **520,** 351–353
99. Reilly, M.A., Dunlop, D.S., and Lajtha, A. (1993). Dietary phenylalanine: does it affect brain function? *J. Neurochem.* **61,** S102
100. Birch, L.L., McPhee, L., and Sullivan, S. (1988). Children's food intake following drinks sweetened with sucrose or aspartame: time course effects. *Physiol. Behav.* **45,** 387–395
101. Tordoff, M.G. and Alleva, A.M. (1990). Effect of drinking soda sweetened with aspartame or high-fructose corn syrup on food intake and body weight. *Am. J. Clin. Nutr.* **51,** 963–969
102. Rodin, J. (1990). Comparative effects of fructose, aspartame, glucose, and water preloads on calorie and macronutrient intake. *Am. J. Clin. Nutr.* **51,** 428–435
103. Rolls, B.J., Kim, S., and Fedoroff, I.C. (1990). Effects of drinks sweetened with sucrose or aspartame on hunger, thirst and food intake in men. *Physiol. Behav.* **48,** 19–26
104. Carlson, H.E. and Shah, J.H. (1989). Aspartame and its constituent amino acids: effects on prolactin, cortisol, growth hormone, insulin, and glucose in normal humans. *Am. J. Clin. Nutr.* **49,** 427–432
105. Fernstrom, J.D. (1988). Carbohydrate ingestion and brain serotonin synthesis: relevance to a putative control loop for regulating carbohydrate ingestion, and effects of aspartame consumption. *Appetite* **11,** 35–41 (suppl 1)
106. Battistin, L., Grynbaum, A., and Lajtha, A. (1971). The uptake of various amino acids by the mouse brain in vivo. *Brain Res.* **29,** 85–89
107. Toth, E. and Lajtha, A. (1981). Elevation of cerebral levels of nonessential amino acids in vivo by administration of large doses. *Neurochem. Res.* **6,** 1303–1320
108. Campistron, G., Guiraud, R., Cros, J., and Mosser, J. (1982). Pharmacokinetics of arginine and aspartic acid administered simultaneously in the rat: I. Plasma kinetics. *Eur. J. Drug Metab.* **7,** 307–313
109. Pitkin, R.M. (1984). Aspartame ingestion during pregnancy. In *Aspartame Physiology and Biochemistry,* (L.D. Stegink and L.J. Filer, Jr., eds.), p. 555–563, Marcel Dekker, New York, NY USA
110. Tobey, N.A. and Heizer, W.D. (1986). Intestinal hydrolysis of aspartylphenylalanine, the metabolic product of aspartame. *Gastroenterology* **91,** 931–937
111. Monte, W.C. (1984). Aspartame: methanol and the public health. *J. Appl. Nutr.* **36,** 42–54
112. Wucherpfennig, K., Dietrich, H., and Bechtel, J. (1983). Alcohol: actual, total and potential methyl alcohol of fruit juices. *Fluess Obst.* **8,** 348–354
113. McMartin, K.E., Makar, A.B., Martin, G., Palese, A.M., and Tephly, T.R. (1975). Methanol poisoning I. The role of formic acid in the development of metabolic acidosis in the monkey and the reversal by 4-methylpyrazole. *Biochem. Med.* **13,** 319–333
114. Kruse, J. (1992). Methanol poisoning. *Intensive Care Med.* **18,** 391–397
115. Tephly, T.R., Green, M.D., and Gamble, J. (1992). Formate metabolism in micropigs. *Toxicol. Appl. Pharmacol.* **116,** 142–145
116. Shahangian, S., Ash, K.O., and Rollins, D.E. (1984). Aspartame not a source of formate toxicity. *Clin. Chem.* **30,** 1264–1265
117. Stegink, L.D., Brummel, M.C., McMartin, K., Martin-Amat, G., Filer, L.J., Jr., Baker, G.L., and Tephly, T.R. (1981). Blood methanol concentrations in normal adult subjects administered abuse doses of aspartame. *J. Toxicol. Environ. Health* **7,** 281–290
118. Andrews, L.S. and Snyder, R. (1986). Toxic effects of solvents and vapors. In *Casarett and Doull's Toxicology: The Basic Science of Poisons,* (L.J. Cuserett, H. Doull, C.D. Klaassen and M.O. Amdur, eds.) p. 636–668, Macmillan Publishing, New York, NY USA
119. Frey, G.H. (1976). Use of aspartame by apparently healthy children and adolescents. *J. Toxicol. Environ. Health* **2,** 401–415
120. Davoli, E., Cappellini, L., Airoldi, L., and Fanelli, R. (1986). Serum methanol concentrations in rats and in men after a single dose of aspartame. *Food Chem. Toxicol.* **24,** 187–189
121. Methanol (1988). In *Medical Toxicology: Diagnosis and Treatment of Human Poisoning,* (M.J. Ellenhorn, D.G. Barceloux eds.) p. 801–805, Elsevier, New York, NY USA
122. Lipton, W.E., Li, Y.-N., Younoszai, M.K., and Stegink, L.D. (1991). Intestinal absorption of aspartame decomposition products in adult rats. *Metabolism* **40,** 1337–1345
123. Kotsonis, F.N. (1986). Toxicology studies done with beta aspartame. In *International Aspartame Workshop Proceedings,* November 17–21, 1986, Marbella, Spain, International Life Sciences Institute Nutrition Foundation, Washington, DC USA
124. Lawrence, J.F. and Iyengar, J.R. (1987). Liquid chromatographic determination of beta-aspartame in diet soft drinks, beverage powders and pudding mixes. *J. Chromatogr.* **404,** 261–266
125. Stamp, J.A. and Labuza, T.P. (1989). Mass spectrometric determination of aspartame decomposition products. Evidence for B-isomer formation in solution. *Food Addit. Contam.* **6,** 397–414
126. Hattan, D. (1988). Toxicological and nutritional evaluation of low nutrient density foods. In *Nutritional and Toxicological Aspects of Food Processing,* (R. Walker, and E. Quattrucci, eds.) p. 275–291, Taylor & Francis, New York, NY USA
127. Burton, E.G., Dal Monte, P., Spears, C., Frank, P., and Oppermann, J.A. (1984). Absorption by the rhesus monkey of phenylalanine methyl ester and species differences in its metabolism by blood plasma and intestinal mucosa. *J. Nutr.* **114,** 1940–1945
128. Camfield, P.R., Camfield, C.S., Dooley, J.M., Gordon, K., Jollymore, S., and Weaver, D.F. (1992). Aspartame exacerbates EEG spike-wave discharge in children with generalized absence epilepsy: A double-blind controlled study. *Neurology* **42,** 1000–1003
129. Benninger, C., Matthis, P., de Sonneville, L.M.J., Lanz-Englert, B., Trefz, F.K., and Bickel, H. (1991). High dose aspartame has no effect on EEG spectral parameters in phenylketonuric heterozygotes (PKUH). *Soc. Neurosci. Abstr.* **17,** 504

130. Wolraich, M.L. (1988). Aspartame and behavior in children. In *Dietary Phenylalanine and Brain Function*, (R.J. Wurtman and E. Ritter-Walker eds.), p. 201–206, Birkhauser, Boston, MA USA
131. Matalon, R., Michals, K., Sullivan, D., Wideroff, L., and Levy, P. (1988). Aspartame consumption in normal individuals and carriers for phenylketonuria. In *Dietary Phenylalanine and Brain Function*, (R.J. Wurtman and E. Ritter-Walker, eds.) p. 41–52, Birkhauser, Boston, MA USA
132. Stegink, L.D., Filer, L.J., Jr., and Baker, G.L. (1981). Plasma and erythrocyte concentrations of free amino acids in adult humans administered abuse doses of aspartame. *J. Toxicol. Environ. Health* **7,** 291–305
133. Stegink, L.D., Filer, L.J., Jr., Baker, G.L., and McDonnell, J.E. (1980). Effect of an abuse dose of aspartame on plasma and erythrocyte levels of amino acids in phenylketonuric heterozygous and normal adults. *J. Nutr.* **110,** 2216–2224
134. Burns, T.S., Stargel, W.W., Tschanz, C., Kotsonis, F.N., and Hurwitz, A. (1991). Aspartame and sucrose produce a similar increase in the plasma phenylalanine to large neutral amino acid ratio in healthy subjects. *Pharmacology* **43,** 210–219
135. World Health Organization/Food and Agriculture Organization Expert Committee. (1988). Energy and Protein Requirements: WHO Technical Report Series No. 724. Geneva, 1985. In *Modern Nutrition in Health and Disease*, 7th edition, (M.E. Shils and V.R. Young, eds.) p. 29, Lea and Febiger, Philadelphia, PA USA
136. Pinto, J.M.B. and Maher, T.J. (1988). Administration of aspartame potentiates pentylenetetrazole- and fluorothyl-induced seizures in mice. *Neuropharmacology* **27,** 51–55
137. Guiso, G., Caccia, S., Vezzani, A., Stasi, M.A., Salmona, M., Romano, M., and Garattini, S. (1988). Effect of aspartame on seizures in various models of experimental epilepsy. *Toxicol. Appl. Pharmacol.* **96,** 485–493
138. Dailey, J.W., Lasley, S.M., Burger, R.L., Bettendorf, A.F., Mishra, P.K., and Jobe, P.C. (1991). Amino acids, monoamines and audiogenic seizures in genetically epilepsy-prone rats: effects of aspartame. *Epilepsy Res.* **8,** 122–133
139. Tilson, H.A., Thai, L., Zhao, D., Sobotka, T.J., and Hong, J.S. (1989). Oral administration of aspartame is not proconvulsant in rats. *Neurotoxicology* **10,** 229–238
140. Zhi, J.Q. and Levy, G. (1989). Aspartame and phenylalanine do not enhance theophylline-induced seizures in rats. *Res. Commun. Chem. Pathol. Pharm.* **66,** 171–174
141. Dailey, J.W., Lasley, S.M., Mishra, P.K., Bettendorf, A.F., Burger, R.L., and Jobe, P.C. (1989). Aspartame fails to facilitate pentylenetetrazol-induced convulsions in CD-1 mice. *Toxicol. Appl. Pharmacol.* **98,** 475–486
142. Cain, D.P., Boon, F., and Bevan, M. (1989). Failure of aspartame to affect seizure susceptibility. *Neuropharmacology* **28,** 433–435
143. Diomede, L., Romano, M., Guiso, G., Caccia, S., Nava, S., and Salmona, M. (1991). Interspecies and interstrain studies on the increased susceptibility to metrazol-induced convulsions in animals given aspartame. *Food Chem. Toxicol.* **29,** 101–106
144. Meldrum, B.S., Nanji, N., and Cornell, R.G. (1989). Lack of effect of aspartame or L-phenylalanine on photically-induced myoclonus in the baboon, Papio papio. *Epilepsy Res.* **4,** 1–7
145. Holder, M.D. (1989). Effects of perinatal exposure to aspartame on rat pups. *Neurotoxicol. Teratol.* **11,** 1–6
146. Sze, P.Y. (1989). Pharmacological effects of phenylalanine on seizure susceptibility: an overview. *Neurochem. Res.* **14,** 103–111
147. Fisher, R.S. (1989). Aspartame, neurotoxicity, and seizures: a review. *J. Epilepsy* **2,** 55–64
148. Shaywitz, B.A., Novotny, E.J., Ebersole, J.S., Anderson, G.M., Sullivan, C.M., and Gillespie, S.M. (1992). Aspartame does not provoke seizures in children with epilepsy. *Pediat. Res.* **31** (4/Part 2), 354A
149. Rowan, A.J. and Shaywitz, B.A. (1991). Aspartame has no effect on seizure incidence or EEG epileptiform discharges in children and adults who reportedly had seizures due to aspartame consumption. *Abstr. Am. Electroencephalographic Soc.* **A104,** 47–48
150. Shaywitz, B.A., Gillespie, S.M., and Shaywitz, S.E. (1988). Effects of aspartame on seizures in children. In Dietary Phenylalanine and Brain Function (R.J. Wurtman and E. Ritter-Walker eds.) p. 163–168, Birkhauser, Boston.
151. de Sonneville, L.M.J., Lanz-Englert, B., Trefz, F.K., Benninger, C., Matthis, P., and Bickel, H. (1991). Absence of neurobehavioral effects of high dose aspartame in phenylketonuric heterozygotes (PKUH). *Abstr. Soc. Neurosci.* **17,** 1233
152. Browne, T.R., Dreifuss, F.E., Penry, J.K., Porter, R.J., and White, B.G. (1983). Clinical and EEG estimates of absence seizure frequency. *Arch. Neurol.* **40,** 469–472
153. Guey, J., Bureau, M., Dravet, C., and Roger, J. (1969). A study of the rhythm of petit mal absences in children in relation to prevailing situations. *Epilepsia* **10,** 441–451
154. Shaywitz, B.A. and Novotny, E.J., Jr. (1993). Aspartame and seizures. *Neurology* **43,** 630–631
155. Camfield, P., Camfield, C., Gordon, K., Dooley, J., and Jollymore, S. (1993). Aspartame and Seizures. Reply from the authors. *Neurology* **43,** 630–631
156. Kim, K.C., Tasch, M.D., and Kim, S.H. (1988). The effect of aspartame on 50% convulsion doses of lidocaine. In *Dietary Phenylalanine and Brain Function*, (R.J. Wurtman and E. Ritter-Walker, eds.) p. 127–130, Birkhauser, Boston, MA USA
157. Lieberman, H.R., Caballero, B., Emde, G.G., and Bernstein, J.G. (1888). The effects of aspartame on human mood, performance, and plasma amino acid levels. In *Dietary Phenylalanine and Brain Function*, (R.J. Wurtman and E. Ritter-Walker eds.) p. 196–200, Birkhauser, Boston, MA USA
158. Elsas, L.J. II and Trotter, J.F. (1988). Changes in physiological concentrations of blood phenylalanine produce changes in sensitive parameters of human brain function. In *Dietary Phenylalanine and Brain Function*, (R.J. Wurtman and E. Ritter-Walker eds.) p. 187–195 Birkhauser, Boston, MA USA
159. Lapierre, K.A., Greenblatt, D.J., Goddard, J.E., Harmatz, J.S., and Shader, R.I. (1990). The neuropsychiatric effects of aspartame in normal volunteers. *J. Clin. Pharmacol.* **30,** 454–460
160. Ryan-Harshman, M., Leiter, L.A., and Anderson, G.H. (1987). Phenylalanine and aspartame fail to alter feeding behavior, mood and arousal in men. *Physiol. Behav.* **39,** 247–253
161. Stokes, A.F., Belger, A., Banich, M.T., and Taylor, H. (1991). Effects of acute aspartame and acute alcohol ingestion upon the cognitive performance of pilots. *Aviat. Space Environ. Med.* **62,** 648–653
162. Saravis, S., Schachar, R., Zlotkin, S., Leiter, L.A., and Anderson, G.H. (1990). Aspartame: effects on learning, behavior, and mood. *Pediatrics* **86,** 75–83
163. Kruesi, M.J.P., Rapoport, J.L., Cummings, E.M., Berg, C.J., Ismond, D.R., Flament, M., Yarrow, M., and Zahn-Waxler, C. (1987). Effects of sugar and aspartame on aggression and activity in children. *Am. J. Psychiat.* **144,** 1487–1490
164. Spiers, P., Schomer, D., Sabounjian, L.A., Lieberman, H., Wurtman, R., Duguid, J., McCarten, R., and Lyden, M. (1988). Aspartame and human behavior: cognitive and behavioral observations. In *Dietary Phenylalanine and Brain Function*, (R.J. Wurtman and E. Ritter-Walker eds.) p. 169–178, Birkhauser, Boston, MA USA
165. Naughten, E.R. (1989). Continuation vs discontinuation of diet in phenylketonuria. *Eur. J. Clin. Nutr.* **43,** 7–12
166. Mabry, C.C. (1991). Status report on phenylketonuria treatment: 1990. *AJDC* **145,** 33
167. Lenke, R.R. and Levy, H.L. (1980). Maternal phenylketonuria and hyperphenylalaninemia: an international survey of the outcome of untreated and treated pregnancies. *N. Engl. Med.* **303,** 1202–1208
168. Smith, I., Lobascher, M.E., Stevenson, J.E., Wolff, O.H., Schmidt, H., Grubel-Kaiser, S., and Bickel, H. (1978). Effect of stopping low-phenylalanine diet on intellectural progress of children with phenylketonuria. *Brit. Med. J.* **2,** 723–726
169. Azen, C.G., Koch, R., Gross-Friedman, E., Berlow, S., Coldwell, J., Krause, W., Matalon, R., McCabe, E., O'Flynn, M., Peterson, R., Rouse, B., Scott, C.R., Sigman, B., Valle, D., and Warner, R. (1991). Intellectual development in 12-year-old children treated for phenylketonuria. *AJDC* **145,** 35–39
170. Legido, A., Tonyes, L., Carter, D., Schoemaker, A., Di George, A., and Grover, W.D. (1993). Treatment variables and intellectual outcome in children with classic phenylketonuria. A single-center-based study. *Clin. Pediat.* **32,** 417–425
171. Rylance, G. (1989). Outcome of early detected and early treated phenylketonuria patients. *Postgrad. Med. J.* **65,** 7–9
172. Thompson, A.J., Smith, I., Brenton, D., Youl, B.D., Rylance, G., Davidson, D.C., Kendall, B., and Lees, A.J. (1990). Neurological deterioration in young adults with phenylketonuria. *Lancet* **336,** 602–605