Carnosine and Its Possible Roles in Nutrition and Health

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Contents

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Abstract   The dipeptide carnosine has been observed to exert antiaging activity at cellular and whole animal levels. This review discusses the possible mechanisms by which carnosine may exert antiaging action and considers whether the dipeptide could be beneficial to humans. Carnosine's possible biological activities include scavenger of reactive oxygen species (ROS) and reactive nitrogen species (RNS), chelator of zinc and copper ions, and antiglycating and anticross-linking activities. Carnosine's ability to react with deleterious aldehydes such as malondialdehyde, methylglyoxal, hydroxynonenal, and acetaldehyde may also contribute to its protective functions. Physiologically carnosine may help to suppress some secondary complications of diabetes, and the deleterious consequences of ischemic—reperfusion injury, most likely due to antioxidation and carbonyl-scavenging functions. Other, and much more speculative, possible functions of carnosine considered include transglutaminase inhibition, stimulation of proteolysis mediated via effects on proteasome activity or induction of protease and

stress-protein gene expression, upregulation of corticosteroid synthesis, stimulation of protein repair, and effects on ADP-ribose metabolism associated with sirtuin and poly-ADP-ribose polymerase (PARP) activities. Evidence for carnosine's possible protective action against secondary diabetic complications, neurodegeneration, cancer, and other age-related pathologies is briefly discussed.

I. INTRODUCTION

Carnosine (β-alanyl-L-histidine) and related compounds, homocarnosine and anserine, together with N-acetylated forms (see Fig. 3.1 for structures), are common dipeptides found in mammals, birds, and fish (Abe, 2000; Bonfanti et al., 1999; de Marchis et al., 2000; Lamas et al., 2007; Tsubone et al., 2007). One remarkable feature of these compounds is that they are often found at relatively high concentrations (Table 3.1). The highest value reported for terrestrial mammals is that of the middle gluteal muscle of the thoroughbred racehorse which contained over 100 mmol of carnosine per kg dry weight of muscle (Dunnet and Harris, 1997). It has recently been reported, however, that the carnosine plus anserine levels in turkey breast muscle can exceed 200 mM (Jones et al., 2007).

It is a valid generalization that there is more carnosine in anaerobic, glycolytic, white muscle than in red, aerobic, muscle (Table 3.2).
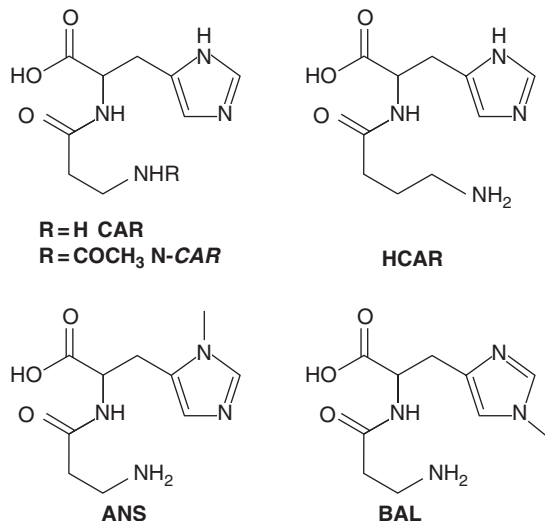
FIGURE 3.1 Structures of carnosine (CAR), N-acetylcarnosine (N-CAR), homocarnosine (HCAR), anserine (ANS), and balanine (BAL).

TABLE 3.1 Carnosine and anserine concentrations in common animal species (from Aristoy and Toldra, 2004)

		Carnosine (mg/100 g)	Anserine (mg/100 g)
Pig	Loin	313	14.5
	Ham	449	22.9
	Neck	186	10.7
Beef	Loin	375	59.7
	Neck	201	25.4
Lamb	Shoulder	39.3	31.5
	Neck	94.2	119.5
Chicken Pectoral		180	772
	Leg	63	233
Turkey	Wing	66	775
Salmon		0.53	589
Trout		1.6	344
Sardine		0.1	1.33

TABLE 3.2 Carnosine content varies according to tissue (from Purchas *et al.*, 2004 and Cornet and Bousset, 1999)

Animal tissues/muscle		Carnosine (mg/100 g)	Anserine (mg/100 g)
Beef	Cheek	42.9	
	Heart	32.6	
	Liver	77.5	
	Semitendinosus muscle	452	
Lamb	Longissimus muscle	491	
	Semitendinosus muscle	356	
	Triceps brachii muscle	251	
Pig	Massetuer muscle	38	6
	Trapezius muscle	147	6
	Longissimus dorsi muscle	268	6

Carnosine is also associated with nervous tissues, including the brain, where it is concentrated especially in the olfactory lobe (Bonfanti *et al.*, 1999; de Marchis *et al.*, 2000). However, human cerebral spinal fluid contains homocarnosine but no carnosine (Huang *et al.*, 2005).

II. CARNOSINE METABOLISM

Carnosine is synthesized from β-alanine and L-histidine by the enzyme carnosine synthase, a reaction which also requires ATP. Studies using primary cell culture have indicated that the dipeptide is synthesized by muscle cells, glial cells and oligodendrocytes (Bauer, 2005). Although carnosine is found enriched in neurons, especially those of the olfactory lobe, it appears that these cells are capable of taking up the dipeptide following its release from glial cells in which it is synthesized. Not unexpectedly, carnosine synthesis is subject to some form of metabolic regulation; synthesis of the dipeptide is reduced when astroglia-rich cultures are treated with dibutyryl cyclic AMP and other agents which activate cyclic AMP-dependent protein kinases (Schulz et al., 1989). It has been suggested that changes in carnosine synthesis accompany morphological differentiation in muscle and astroglia (Bauer, 2005).

Carnosine can be acetylated at its amino terminus to form N-acetyl-carnosine, although the enzyme responsible has not been characterized. It has also been reported that N-acetyl-carnosine is readily de-acetylated in the tissues (Barbizhayev, 2008). A phosphorylated form of carnosine has also been described (Quinn et al., 1992), but again little more is known about the enzymes responsible or its function.

III. CARNOSINE AND NEUROLOGICAL ACTIVITY

Animal studies have shown that carnosine can affect neurological function, not surprising given that fact the dipeptide is synthesized by the brain and that specific transporters for it are present in the choroid plexus (Teuscher et al., 2004), part of the blood–brain barrier. One possible role for carnosine within the neuronal system is modulation in glutamatergic sensory neurons (Bonfanti et al., 1999). For a detailed discussion of carnosine's function within the mammalian brain, the reader is referred to the fine review by Bonfanti et al. (1999).

The kidney brush border also possesses a carnosine transport system and there is evidence that kidney also contains an active carnosinase (Sauerhoefer et al., 2005). There is also evidence that carnosine can influence sympathetic nervous activity in kidney (Tanida et al., 2005) as well as brown (Tanida et al., 2007) and white adipose tissue (Shen et al., 2008). Other studies have shown that carnosine has antidepressant activity in rats (Tomonaga et al., 2008). In chicks, carnosine induces hyperactivity (Tsuneyoshi et al., 2007) whereas its reverse structure (L-histidinyl-β-alanine) has sedative and hypnotic effects (Tsuneyoshi et al., 2008). The mechanisms involved in remain obscure however.

IV. CARNOSINE AND OTHER TISSUES

Although carnosine seems to be primarily associated with the brain and innervated tissues such as muscles (skeletal and heart) at least in the rat (Aldini *et al.*, 2004), carnosine has been reported to be present in the eye lens (Quinn *et al.*, 1992), which suggests that its function might not be restricted nervous tissue.

V. POSSIBLE FUNCTIONS OF CARNOSINE

Although carnosine was discovered over 100 years ago, much remains to be revealed about its functions; indeed carnosine and homocarnosine have been described as forgotten and enigmatic dipeptides (Bauer, 2005). There are numerous examples of protective actions of carnosine against a variety of insults mediated by discrete entities (oxygen free radicals, reactive nitrogen species, glycating agents, deleterious alde-hydes, toxic metal ions) as well as ameliorating conditions associated with aging. Carnosine has been shown to protect various cells against ischemia–reperfusion injury, for example in rat liver (Fouad *et al.*, 2007; Fujii *et al.*, 2003), kidney (Kurata *et al.*, 2006), heart (Alabovsky *et al.*, 1997; Lee *et al.*, 1999; Zaloga and Siddiqui, 2004), and brain (Dobrota *et al.*, 2005). Protective activity exerted by carnosine has also been observed with respect to diabetes, osteoporosis, neurodegeneration, wound healing and loss of vision, and hearing and immune function.

Possible biochemical functions (Quinn *et al.*, 1992) of carnosine include control of pH, immunostimulant, wound healing agent, antioxidant, metal-ion chelator, carbonyl scavenger, and antiglycator (Table 3.3). The evidence for some of these proposals is highly varied, however.

VI. CONTROL OF pH

The most convincing proposal is that carnosine plays one or more roles in control of intracellular hydrogen ion concentration (Abe, 2000; Vaughan-Jones *et al.*, 2006). Carnosine is an effective physiological buffer; it is presumed that this property explains its predominant association with white, glycolytic, muscles which possess relatively few mitochondria and thereby generate lactic acid. Not only may carnosine, also possible in its acetylated form, help to directly suppress the rise in hydrogen ion con-centration but its ability to activate the enzyme carbonic anhydrase (Temperini *et al.*, 2005) would increase bicarbonate buffer capacity. These properties may help explain carnosine's protective action in ischae-mia, a condition associated with severe intracellular acidosis.

TABLE 3.3   Possible Homeostatic properties of carnosine

- Buffer
- Hydroxyl radical scavenger
- Antioxidant
- Chelator of copper and zinc ions
- Aldehyde/carbonyl scavenger
- Antiglycator
- Stimulates nitric oxide synthesis
- Stimulates proteolysis
- Activates carbonic anhydrase
- Upregulates synthesis of oxidized protein hydrolase (OPH)
- Suppresses protein cross-linking
- Reacts with protein carbonyls
- Suppresses AGE reactivity
- May participate in protein deglycation
- May participate in histone deacetylation
- May participate in repair of isoaspartate residues
- May stimulate synthesis of stress proteins

VII. CARNOSINE AND CHELATION OF ZINC AND COPPER IONS

Carnosine is an avid chelator of metal ions (Baran, 2000). Complexes with calcium, copper, and zinc ions have been described (Trombley *et al.*, 2000). It is possible, therefore, that carnosine could exert some sort of control of calcium metabolism in muscle tissue (heart or skeletal). It is also likely that the dipeptide controls the availability of zinc ions in neuronal tissue, especially the olfactory lobe where both carnosine and zinc are enriched (Bakardjiev, 1997; Bonfanti *et al.*, 1999; Sassoe-Pognetto *et al.*, 1993). Zinc–carnosine complexes, called polaprezinc, are also effective in the repair of ulcers and other lesions in the alimentary tract (Matsukura and Tanaka, 2000).

VIII. CARNOSINE AND AGING

It has been previously suggested that carnosine might possibly be an antiaging agent (Boldyrev *et al.*, 1999a; Hipkiss, 1998; Hipkiss *et al.*, 2001). This suggestion was based on (i) a report of observations made in Australia around 1990, but finally published in 1994, that carnosine could

not only delay senescence in cultured human fibroblasts but also reverse the senescent phenotype by promoting what appeared to be rejuvenating effects (McFarland and Holliday, 1994, 1999), and (ii) a substantial body of work from Russian laboratories in the 1980s and 1990s on the dipeptide's antioxidant activity and protective functions towards heart and kidney ischaemia (see Boldyrev, 1993; Boldyrev et al., 1993, 1995, 1997; Quinn et al., 1992; and references therein). Later work showed that growing fibroblasts with carnosine also protected telomeres against shortening (Shao et al., 2004). Antiaging effects of carnosine were subsequently observed in senescence-accelerated mice and fruit flies (Yuneva et al., 1999, 2002). That carnosine seems to be specifically associated with long-lived, postmitotic tissue, such as muscle and nerves, is at least consistent with the idea that the dipeptide does not compromise cell survival and may help ensure longevity.

IX. CARNOSINE AND THE CAUSES OF AGING

The explanation of the causes of aging remains somewhat controversial. Most, but not all, biogerontologists reject the idea that aging is a genetically programed process along the lines of growth and development, because the majority of animals in the wild die from predation, starvation or disease, before they age significantly. Hence the selection of genes specifically programing aging would not be of any evolutionary advantage. The consensus of opinion is that aging is the result of a breakdown of molecular homeostasis, due to the chronic effects of the forces of instability (endogenous and exogenous) to which all cells and organisms are continuously subjected. In other words, organisms have evolved to survive long enough to reproduce their genes, during which time entropic events must be either controlled or their effects eliminated. Consequently, the changes which we refer to as aging are thought to have no evolutionary significance, but result from the eventual failure of longevity genes whose functions ensure survival for sufficient time for the organism to reproduce successfully. That there is a frequent correlation between organism longevity and the ability to resist certain external stresses, such as heat or irradiation, is consistent with this idea.

Various possible mechanisms have been proposed to explain aging. These include environmental and endogenous factors which affect an organism's ability to survive by causing genetic changes (e.g., DNA damage and telomere shortening), altering gene expression, increasing oxidative stress, compromising energy provision and promoting the

accumulation of altered proteins. There is evidence which suggests that carnosine has at least the potential to ameliorate to some degree most of these possible causes of aging (Hipkiss, 1998).

X. PROTEOTOXICITY AND AGING

At a biochemical level the most common symptom of aging and its related pathologies is the accumulation of altered or abnormal proteins (Dalle-Donne *et al.*, 2003; Hipkiss, 2006a; Levine, 2002). It should be pointed out that abnormal proteins are normally formed continuously, intracellulary, and extracellularly, and they originate from biosynthetic errors (gene expression is not 100% perfect) and postsynthetic damage due to the actions of deleterious endogenous and exogenous agents (e.g., oxygen and glucose). It is generally thought that the age-related accumulation of aberrant polypeptides is a consequence of the decline in functional activity of the gamut of homeostatic process (e.g., DNA repair, proteolysis, antioxidant enzymes), because the molecules which carry out these fun ctions are themselves also subjected to the same range of insults which they should normally prevent or eliminate. Indeed it is becoming increasingly apparent that molecular overengineering of certain of these homeostatic gene products can indeed increase both stress-resistance and organism longevity. Furthermore, not only does the accumulation of aberrant polypeptides result in loss of function of the normal gene products, but these altered molecules also appear to possess gain of function toxicity, mostly due to their aggregation, oligomerization, and cross-linking potential. Certain aberrant proteins also induce oxygen free radical generation. Among the better understood resultant effects of altered protein accumulation are compromised proteolytic activities, inflammation (or sometimes called ''inflammaging''), and induction of the stress response. An obvious contentious question is: does aging causes proteotoxocity, or does proteotoxicity causes aging? Most likely the answer is ''yes'' to both alternatives, simply because proper control of protein metabolism (synthesis and degradation) is essential for viability.

Table 3.4 lists the possible areas in which carnosine could theoretically exert some protective, homeostatic effects which suppress cellular and/or organism aging, by the dipeptide mostly acting at the postsynthetic level to suppress formation of altered proteins. However, it is also possible that, by scavenging oxidative and glycoxidative agents, carnosine could inhibit gene modification and thereby prevent synthesis of altered gene products and general DNA damage.

TABLE 3.4   Hypothetical mechanisms of carnosine's antiaging activity

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Suppresses altered protein accumulation by:
- Scavenging hydroxyl radicals and hypochlorite ions
- Scavenging endogenous and exogenous toxic aldehydes and glycating agents
- Reacting with protein carbonyls/AGEs
- Inhibiting transglutaminase activity
- Slowing protein synthesis

Activates altered protein elimination by:
- Stimulating expression of oxidized protein hydrolase
- Stimulating NO synthesis which upregulates proteasome activity
- Stimulating synthesis of stress/chaperone proteins
- Participating in protein deglycation

Participation in SIRT metabolism (gene silencing) by:
- Accepting acetyl groups released by SIRT-mediated histone deacetylation
- Scavenging (acetyl)-ADP-ribose units generated following NAD cleavage which accompanies SIRT-mediated histone deacetylation

Protects telomeres against shortening
Upregulates release of corticosterone

XI. CARNOSINE, OXYGEN FREE RADICALS, AND OXIDATIVE STRESS

Over 50 years ago Harman (1956) proposed the so-called ''oxygen free radical theory of aging.'' This theory proposed that much age-related damage to proteins, lipids, and DNA was caused by incompletely reduced oxygen atoms, that is, oxygen free radicals. It has often been assumed that mitochondria are the principle source of these reactive oxygen species (ROS) because of the organelles' intimate association with oxygen. However, it should be noted that ROS can be produced elsewhere within the cytosol and extracellularly too. ROS are not exclusively deleterious as they are also involved in cell signaling, although there are enzymes such as superoxide dismutase, catalase, and various peroxidases, in the cytosol and mitochondria, which provide defense against excessive ROS generation. There is evidence that carnosine can also exert antioxidant activity inhibiting oxidation of lipids (Bogardus and Boissonneault, 2000; Decker *et al.*, 2001; Nagasawa *et al.*, 2001) and proteins (Boldyrev *et al.*, 1999a,b; Guiotto *et al.*, 2005a; Hipkiss *et al.*, 1998a;

Kang *et al.*, 2002; Kim and Kang, 2007; Quinn *et al.*, 1992). The dipeptide can scavenge hydroxyl radicals (Tamba and Torreggiani, 1999), which are the most damaging ROS. Hydroxyl radicals are generated from hydrogen peroxide in the presence of bivalent metal ions such as copper; hydrogen peroxide is formed by the action of superoxide dismutase on superoxide anions. Carnosine can also scavenge at least two other deleterious ROS, the hypochlorite anion (OCl^-) (Hipkiss *et al.*, 1998a; Quinn *et al.*, 1992), which is formed from superoxide and chlorine ions by the action of myeloperoxidase, and peroxynitrite ($ONOO^-$) (Fontana *et al.*, 2002) which is formed by the reaction of superoxide with nitric oxide. Consequently, it is theoretically possible that carnosine could prevent damage mediated by these ROS *in vivo*.

XII. CARNOSINE AND NONENZYMIC PROTEIN GLYCOSYLATION (GLYCATION)

Other sources of age-related macromolecular damage are metabolic aldehydes and ketones. And the best investigated example is the chemical process, originally described in cooking, called the Maillard or browning reaction. This process, originally termed nonenzymic protein glycosylation, but now called glycation, involves the reaction of a reducing sugar such as glucose with an amino group of a protein, eventually producing a highly complex brown product, now known as advanced glycation endproducts (AGEs) (Suji and Sivakami, 2004). In fact, it turns out that glucose is the least reactive of all the common metabolic sugars due to the fact that its aldehyde group is 99.99% unavailable for reactivity because of the predominant cyclic form of the glucose molecule. Other common sugars such as galactose and fructose are much more reactive than glucose; indeed diets high in galactose and fructose are frequently employed experimental tools to induce diabetes-like symptoms in laboratory animals (Wang *et al.*, 2008). It has also been found that certain metabolic intermediates and their by-products, if present in excess, can glycate proteins (Brownlee, 1995; Thornalley, 1999), DNA (Barea and Bonatto, 2008), and amino lipids (Lankin *et al.*, 2007) very rapidly indeed to generate products very similar to those found in senescent cells and organisms.

  As described above, carnosine was shown in the 1990s to exert antiaging effects in cultured cells, and the question arose about the mechanism (s) involved. When this work was initiated, carnosine was usually regarded as an antioxidant (Kohen *et al.*, 1988) but as other and better antioxidants did not exert the antiaging/rejuvenating effects on cultured fibroblasts, this suggested that additional activities were necessary to explain its actions. It was suggested that carnosine's structure resembled preferred protein glycation sites and it was demonstrated that the

dipeptide did indeed possess antiglycating activity (Hipkiss *et al.*, 1995a). It inhibited protein glycation, subsequent cross-linking (Hipkiss *et al.*, 1998a), and formation of AGEs induced by a variety of reactive aldehyde and carbonyl compounds (glucose, deoxyribose, ribose, fructose, dihydroxyacetone, malondialdehyde, acetaldehyde, formaldehyde, and methylglyoxal) (Brownson and Hipkiss, 2000; Hipkiss and Chana, 1998; Hipkiss *et al.*, 1998b, 2002). These observations have been confirmed and greatly extended by other workers (Burcham and Pyke, 2006; Gugliucci *et al.*, 2002; Seidler, 2000; Seidler *et al.*, 2004; Yan and Harding, 2005; Ukeda *et al.*, 2002; see also references in Hipkiss, 2005), who have detailed the chemistry of the various carnosine–carbonyl adducts generated (Aldini *et al.*, 2002, 2005; Carini *et al.*, 2003; Liu *et al.*, 2003). A carnosine–carbonyl adduct, formed between the dipeptide and hydroxynonenal, has been isolated from oxidatively stressed biological tissue (Orioli *et al.*, 2005) and, furthermore, detected in rat urine (Orioli *et al.*, 2007). Given that tissue levels of carnosine are generally higher in humans than in rodents (Hipkiss and Brownson, 2000), it is anticipated that similar adducts will be detected in human tissues.

Having shown that carnosine can suppress the reactivity of low molecular weight carbonyl compounds by simply reacting with the deleterious glycating agents, we suggested that carnosine could react with carbonyl groups generated on macromolecules such as aminolipids and proteins following oxidation or glycation. Using radiolabeled carnosine, we showed (Brownson and Hipkiss, 2000), at least at the test-tube level, that the dipeptide could indeed react with protein-bound carbonyl groups, and the term ''protein carnosinylation'' was coined. As yet, however, no evidence for the presence of ''carnosinylated'' protein has been obtained from biological tissue. However, protein γ-glutamyl-carnosine adducts have been detected (Kuroda and Harada, 2002) in animal muscle. There are a number of differing explanations that could account for the formation of these adducts. The γ-glutamyl-carnosine adduct may derive from the reaction of carnosine with the transient carbonyl group generated during the spontaneous deamidation of a glutamine residue (Kuroda and Harada, 2002). Another possibility is that adduct formation results from the action of transglutaminase on a protein glutamine residue and carnosine producing a γ-glutamyl-carnosine residue in the protein; subsequent proteolysis would release the free γ-glutamyl-carnosine adduct. A third explanation is the reaction of carnosine with glutamate semialdehyde, formed following ROS-mediated oxidation of a protein arginine residue; subsequent hydrolysis again releasing the free adduct. As noted above, although no ''carnosinylated'' protein has been detected, a ''carnosinylated'' lipid has been detected in human muscle (Schroder *et al.*, 2004), possibly arising from the reaction of an oxidized (amino)-lipid

with carnosine. Indeed, carnosine has been shown to inhibit MG-induced glycation of LDL and formation of foam cells in vitro (Rashid *et al.*, 2007).

There is one study which investigated the effects of carnosine on formation of protein carbonyls in cultured cells following exposure to glucose degradation products present in sterilized peritoneal dialysis fluids. It was shown that preexposure of cultured mesothelial cells to 20 mM carnosine suppressed ROS generation and formation of protein carbonyls induced by the glucose degradation products (Alhamdani *et al.*, 2007a,b). The mechanism by which this protection was exerted remains uncertain, however; possibilities are (i) the extracellular carnosine may simply react with the deleterious carbonyl compounds extracellularly; (ii) intracellular carnosine reacts with the carbonyl compounds thereby preventing their interaction with intracellular macromolecules; and (iii) carnosine reacts with protein carbonyls forming ''carnosinylated'' proteins. This problem should not be difficult to resolve experimentally.

Deleterious protein cross-linking can also be induced by reactive nitrogen species (RNS) such as peroxynitrite ONOO formed by the reaction of superoxide with nitric oxide (NO). The cross-links are formed between tyrosine residues following nitration by peroxynitrite (Sitte, 2003). Carnosine appears to play roles not only in NO generation but also in protection against excess NO production by inducible nitric oxide synthetase (NOS), thereby preventing ONOO-mediated protein modification (Fontana *et al.*, 2002). Evidence for a carnosine–NO adduct has also been published (Nicoletti *et al.*, 2007).

XIII. CARNOSINE AND PROTEOLYSIS OF ALTERED PROTEINS

As noted above, accumulation of altered protein forms is a common feature of aging, which can be explained by either, or both, increased generation of the aberrant polypeptides or a decrease in cellular ability to eliminate them by selective proteolysis (Hipkiss, 2006a). In the past few years, much evidence has emerged showing that cell senescence is accompanied by decreases in either, or both, proteasome- and autophagy-mediated proteolysis (Bergamini *et al.*, 2007; Bulteau *et al.*, 2006; Carrard *et al.*, 2002; Donati, 2006; Martinez-Vicente and Cuevo, 2007; Ngo and Davies, 2007; Vernace *et al.*, 2007a). Indeed, upregulation of either, or both, proteasome and autophagic activity has been shown to delay onset of the senescent state in cultured cells (Bergamini *et al.*, 2007; Chondrogianni and Gonos, 2007; Donati, 2006; Hansen *et al.*, 2008; Vernace *et al.*, 2007b). Quite why these proteolytic activities decline during aging is uncertain; possible explanations include inhibition of proteasome activity by cross-linked proteins (Carrard *et al.*, 2002; Ding and Keller, 2001), and accumulation of lipoprotein cross-linked material

(lipofuscin) in the autophagosomes (Dahlmann, 2007; Tatsuta and Langer, 2008). Also it should be noted that participation of chaperone proteins is necessary for the recognition, delivery, and degradation of altered proteins (Leidhold and Voos, 2007; Otto *et al.*, 2005; Rakwalska and Rospert, 2004). This may help explain why increased expression of certain chaperone proteins can extend life span in some organisms as well as protect against heat and other stressing agents (Liao *et al.*, 2008 Morrow and Tanguay, 2003; Rattan, 2006).

Although carnosine's ability to suppress both formation and reactivity of some of the age-associated macromolecular modifications (e.g., protein–protein cross-links and protein AGEs) could contribute to its apparent antisenescent effects, these prophylactic actions cannot by themselves explain the dipeptide's apparent rejuvenating activity towards cultured human fibroblasts observed by McFarland and Holliday (1994, 1999). It is possible that carnosine may stimulate proteolysis. We obtained preliminary evidence that protein breakdown is increased in ''old'' fibroblasts when cultured with carnosine (Hipkiss *et al.*, 1998b), while Bharadwaj *et al.* (2002) showed that the dipeptide stimulated proteolysis of HIF-1α protein in cultured cardiomyocytes. Carnosine was also shown to stimulate neutral (nonlysosomal) protease activity in cell-free extracts from rat brain (Bonner *et al.*, 1995). Intracellular elimination of aberrant polypeptides mostly, though not exclusively, involves the proteasomes, and recent evidence suggests that nitric oxide can stimulate proteasomal activity (Kotamraju *et al.*, 2006; Thomas *et al.*, 2007). It is possible that carnosine can upregulate NOS as it has been suggested that carnosine itself is the source of NO rather than arginine (Alaghband-Zadeh *et al.*, 2001). Therefore, in addition to decreasing the formation of glycated and cross-linked protein which can inhibit proteasomal activity, carnosine may actually stimulate proteasomal function to improve the elimination altered protein forms. This proposal should be easy to test using cell culture and in tissues of aging animals fed a carnosine-enriched diet.

XIV. CARNOSINE AND GENE EXPRESSION

Carnosine can affect gene expression. Ikeda *et al.* (1999) showed that carnosine markedly upregulates vimentin synthesis in cultured rat fibroblasts, while an association between carnosine and vimentin, a cytoskeletal, intermediate filament protein has been noted in glial cells and neurons (Bonfanti *et al.*, 1999). Interestingly, it has also been shown that the protease, oxidized protein hydrolase (OPH), is coexpressed with vimentin in COS cells (Shimizu *et al.*, 2004). Thus, it is at least possible that carnosine could induce synthesis of OPH in the cultured human fibroblasts and thereby increase the cellular ability to eliminate oxidized

polypeptides. While much more needs to be done to confirm or refute many of these proposals, they could help to explain carnosine's rejuvenating actions of cultured human fibroblasts, particularly as increased protein turnover is a well-recognized antiaging strategy (Hipkiss, 2003). However, it should be noted that excessive proteolysis may contribute to the aging phenotype as in the case of age-related muscle wastage or sarcopenia.

It has recently been reported that vimentin is very readily and specifically glycated in cultured human fibroblasts (Kueper *et al.*, 2007). The biological significance of this observation is at present uncertain but the fact that carnosine seems to mimic preferred glycation sites and apparently promotes expression of a protein (vimentin) which is itself highly susceptible to glycation may not be entirely coincidental and should be explored. Other studies have shown that activated macrophages secrete vimentin, an intermediate filament protein, which may play a role in bacterial killing and generation of oxidative metabolites (Mor-Vaknin *et al.*, 2003). It is possible that the released vimentin helps to quench any excess glycoxidation species that are generated by activated leukocytes.

It has recently been shown that senescent human fibroblasts accumulate a particular stress protein modified by glycation (Unterluggauer *et al.*, 2008). It was found that heat cognate protein Hsc70 appears to be a target for selective glycation in senescent fibroblasts. One conjectures therefore whether the generation of the highly glycated Hsc70 protein (Hsc70AGE) is part of a triggering or sensing mechanism for the induction of stress-induced senescence, and, furthermore, whether carnosine's antisenescence effects might be related to its ready glycation thus sparing the Hsc70 protein from modification.

A form of rejuvenation of the senescent phenotype occurs in phorbol ester-treated U937 leukemia cells and that changes in proteolytic activity and vimentin expression are involved (Hass, 2005). It has been known for some time that the enzyme poly-ADP-ribose-polymerase-1 (PARP-1) plays an important role in suppressing cellular senescence and the response to cellular stress. Recent studies have shown that PARP-1 can associate with and strongly stimulate the 20S proteasomes, an activity which is involved in the selective degradation of oxidized proteins (Selle *et al.*, 2007). It appears that PARP-1 catalyzes the synthesis of poly-ADP-ribose (from NAD^+ units) and becomes attached to various acceptor proteins located in the nucleus. One conjectures whether there is any parallel between the metabolic changes which accompany the rejuvenation phenomenon described above, and carnosine's apparent ability to control vimentin expression, its proposed effects on proteolytic activity, its potential to react with glycating agents such as ADP-ribose, and its ability to induce cellular rejuvenation in cultured human fibroblasts described by McFarland and Holliday (1994, 1999).

XV. CARNOSINE, ANTICONVULSANTS, AND AGING

Another form of protein dysfunction which accompanies aging is the spontaneous deamidation of asparagine residues which can result in the generation of isoaspartate residues in proteins. The enzyme protein-isoaspartate-methyltransferase (PIMT) plays an important role in the repair of isoaspartate residues converting them into the normo-form via formation of a cyclic succinimide intermediate (Zhu et al., 2006). A recent study has shown that hydroxylamine can selectively cleave this inter-mediate generating a normal C-terminal fragment plus an N-terminal fragment with either an aspartyl-N-hydroximide or an aspartyl dihydrox-amate residue at its C-terminal end (Zhu and Aswad, 2007). It is interest-ing that hydroxylamines can, like carnosine, delay senescence in cultured human fibroblasts (Atamna et al., 2000) and react with carbonyl groups (Hipkiss, 2001). Given its basic nature, one therefore speculates whether carnosine could similarly cleave peptide bonds at isoaspartate residues, possible even in vivo, and this action may further contribute to the dipeptide's antiaging activity. Such action could generate carnosine adducted to N- and C-terminal protein fragments which would be easy to detect, should they exist.

Studies in aging models using nematode worms have shown that anticonvulsants (valproic acid, volpramide, trimethadione, and ethosux-imide) extend life span (Evason et al., 2005; Hughes et al., 2007). Some anticonvulsants also upregulate carnosine levels in mouse brain and homocarnosine levels in human brain (Petroff et al., 1998, 2006). Both carnosine and homocarnosine also have anticonvulsant activity in mice, rats, and humans (Jin et al., 2005; Kozan et al., 2008; Petroff et al., 1998; Wu et al., 2006; Zhu et al., 2007). It is also thought, however, that carnosine's anticonvulsant action is exerted via a carnosine–histidine–histamine pathway (Zhu et al., 2007) activating histaminergic, GABAer-gic, and glutamicergic systems (Kozan et al., 2008).

Whether there is any other connection between anticonvulsant activity and carnosine's antiaging actions is obviously highly speculative. It may be relevant to note that epileptic seizures and a shortened life span, together with altered protein accumulation, are consequences of PIMT-deficiency in mice, while treatment with valproic acid, an anticonvulsant, partially suppresses these symptoms including effects on life span (Yamamoto et al., 1998). Conversely, PIMT overexpression can increase life span of Drosophila (Bennet et al., 2003). Furthermore, the chemistry of some anticonvulsants (ethosuximide) resembles quite closely the structure of the succinimide intermediate formed during both aspara-gine deamidation and PIMT-mediated repair of isoaspartate residues. One conjectures whether there are any relationships between these

observations particularly because PIMT levels are 50% downregulated, posttranslationally, in epileptic human hippocampus (Lanthier *et al.*, 2002), leading to the accumulation of aberrant tubulin.

Another speculation which can be offered in this context is whether carnosine stimulates PIMT expression. It is known that small molecules regulate expression of PIMT mRNA. For example, *R*-(−)-deprenyl (Huebscher *et al.*, 1999), lithium, and valproic acid (Lamarre and Desrosiers, 2008) can upregulate PIMT, while the cyclic tripeptide argininyl–glycyl–aspartyl inhibits PIMT expression (Lanthier and Desrosiers, 2006). One wonders therefore whether carnosine is an activator of PIMT expression functioning at either transcriptional or translational levels; both the isoaspartyl residue in the protein to be repaired and carnosine contain β-peptide bonds. It may only be coincidental that PIMT expression decreases with tumor malignancy (Lapointe *et al.*, 2005) and carnosine can inhibit tumor cell growth (Holliday and McFarland, 1996).

The biological role of PIMT involves the selective methylation of isoaspartate residues followed by a demethylation step to reform the succinimide intermediate. The demethylation causes the release of methanol which can be converted to formaldehyde and finally to formic acid, as demonstrated in rat brain preparations. It was found that *S*-adenosylmethionine (SAM), the methyl donor, caused formaldehyde levels to rise in the rat brain homogenates, thus suggesting that excessive formaldehyde may be a precipitating factor in Parkinsons's disease (PD) (Lee *et al.*, 2008). It is possible that carnosine could suppress formaldehyde toxicity by reacting with it to generate a carnosine–formaldehyde adduct. This should be a relatively easy experiment to perform to test this prediction.

XVI. CARNOSINE AND DIETARY RESTRICTION-MEDIATED DELAY OF AGING

There is much evidence that caloric restriction (CR) can delay aging and onset of much age-related pathology in many species, and increase maximum life span (see Partridge and Brand, 2005; and references cited therein). Recent observations suggest that fasting periods, rather than a decrease in overall caloric intake per se, may be the cause of these effects (Goodrick *et al.*, 1990; Mager *et al.*, 2006; Masternak *et al.*, 2005; Mattson and Wan, 2005). The mechanisms involved remain uncertain but they are currently thought to involve an interaction between gene expression and carbohydrate metabolism. Genetic studies have indicated that histone/protein deacetylase (sirtuin) activities have important roles in the CR phenomenon (Guarente, 2000; Westphal *et al.*, 2007). Coupled with the

sirtuin-mediated deacetylation reaction is the conversion of NAD^+ to ADP-ribose and nicotinamide (Lin *et al.*, 2000; Medvedik *et al.*, 2007).

There are a number of theoretical locations where carnosine might have some influence on sirtuin-mediated protein deacetylation and ADP-ribose metabolism. First, carnosine can behave as an acetyl acceptor by forming *N*-acetyl-carnosine. Secondly, ADP-ribose, a product of sirtuin-mediated NAD-coupled protein deacetylation, is a potent glycating agent. Hence carnosine could be the ultimate acceptor for ADP-ribose by generating as adduct, ADP-ribosyl-carnosine. A third possibility concerns the polyADP-ribosylation of certain proteins, as a consequence of oxidative stress, carried out by a PARP, using NAD^+ as the ADP-ribose source. A number of studies have shown that PARP is involved in aging regulation and may protect cells against senescence (Burkle *et al.*, 2005; Hass, 2005). Again, given the glycating potential of ADP-ribose and carnosine's antiglycating and antiaging properties, one speculates on whether the dipeptide plays a role in either the formation or, following proteasomal activity on the poly-ADP-ribosylated proteins (Selle *et al.*, 2007), the subsequent depolymerizition of the modified protein. However, no carnosine–ADP-ribose adduct has been reported.

Another possible explanation of the effects of dietary restriction (DR) on the aging process might involve a decrease in glycolysis which inevitably accompanies DR-induced fasting periods (Hipkiss, 2006b). This could result in a decrease in the production of the highly deleterious glycolytic by-product, methylglyoxal (MG), whose reactivity towards proteins carnosine can inhibit (Brownson and Hipkiss, 2000; Hipkiss and Chana, 1998). It is possible that control of sirtuin activity by NAD^+/NADH levels can also influence generation of altered proteins by increasing or decreasing MG generation. NAD^+ is essential for the conversion of the glycolytic intermediate glyceraldehyde-3-phosphate (G3P) to 1,3-diphosphoglycerate (1,3-DPG) by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) which also yields NADH. In the ad libitum-fed (AL) condition, it is likely that NAD^+ levels would be low and NADH levels high due to continuous glycolysis. This would limit GAPDH activity and promote G3P accumulation, together with its immediate precursor dihydroxyacetone phosphate (DHAP). It is important to note that both G3P and DHAP are very effective glycating agents which can readily modify protein amino groups, etc. More importantly, however, both G3P and DHAP can spontaneously decompose into MG, the exceedingly toxic glycating agent that may also be responsible for much of the protein/lipid glycation observed during hyperglycaemic conditions. Indeed there are a number of observations suggesting that MG can induce ROS production and many of the deleterious physiological and biochemical changes characteristic of the aged phenotype (Cantero *et al.*, 2007; Desai and Wu, 2008; Dhar *et al.*, 2008; Han *et al.*, 2007;

Jia and Wu, 2007; Schalkwijk *et al.*, 2008; Vander Jagt, 2008; Yamawaki *et al.*, 2008; also see Hipkiss, 2006b, 2008a; and references therein). Carnosine's ability to react with MG (Brownson and Hipkiss, 2000; Hipkiss and Chana, 1998) could conceivably contribute to inhibiting the deleterious effects of this highly reactive endogenous glycating agent, especially in tissues where glycolysis is extensive or persistent.

In DR conditions during the fasting periods, NADH would be metabolized by the mitochondria and NAD^+ regenerated, thus allowing G3P oxidation, preventing DHAP build-up and MG production, and thereby decreasing the incidence of protein and lipid glycoxidation. This model (see Fig. 3.2) would also explain the so-called oxygen paradox where increased mitochondrial function (aerobic metabolism) is found to be beneficial with respect to aging and many related conditions (Hipkiss, 2008a).

It is also possible that protein AGEs have a major role in affecting aging in animal models. Cai *et al.* (2008) have recently demonstrated that an oral glycotoxin (protein AGE made by treatment of albumin with MG) can substantially abolish many of the beneficial effects that CR exerts on aging, at least in mice. They found that the presence of the AGE (MG-modified protein) in the diet of CR mice promoted an age-related increase in oxidative stress similar to that observed in animals fed ad libitum; the life span of the AGE-treated mice was also decreased to that observed in the ad libitum-fed mice. It was concluded that as normal laboratory mouse food contains large amounts of protein AGEs, any reduction of food intake will automatically decrease the AGE load. It therefore follows, somewhat controversially, that the explanation of the beneficial effects of CR on organism life span may have little to do with decreased calorie intake, but instead reflects the effects of AGEs, exogenous, and endogenous, via MG generation. It has been proposed (Hipkiss, 2007b, 2008a,b,c) that dietary restriction, induced by CR or intermittent fasting, will decrease MG formation and thereby lower endogenous AGE generation, compared to animals fed ad libitum in which MG levels are likely to be raised due to increased frequency of food intake.

Many other studies have suggested that MG is a major source of metabolically generated AGEs which in turn can affect organism life span, especially as MG can provoke many of the deleterious changes associated with aging (see Hipkiss, 2008a,b,c; and references therein). For example, mutation in the gene coding for triose phosphate isomerase which provokes the accumulation of the MG precursor dihydroxyacetone phosphate, induce a shortened life span in *Drosophila* (Celotto *et al.*, 2006; Gnerer *et al.*, 2006). Defects in the enzyme responsible for the detoxification of MG, glyoxalase-1, shortens life span of the *Caenorhabditis elegans* (Morcos *et al.*, 2008), while overexpression of glyoxalase-1 can extend life span in the nematode (Morcos *et al.*, 2008). There is a substantial body of

Glucose

DHAP → MG

G3P → MG

Glycation ROS

NAD$^+$

NADH

1, 3 DPG → ATP

Pyruvate

Acetyl –CoA

Mitochondrial dysfunction

NADH O_2

ATP

Carnosine

NAD$^+$ CO_2 H_2O

Mitochondrion

Sirtuin activity

MG-carnosine adduct

Nicotinamide

Controls gene expression to maintain juvenile character

A speculation on how carnosine and caloric restriction may modulate ageing
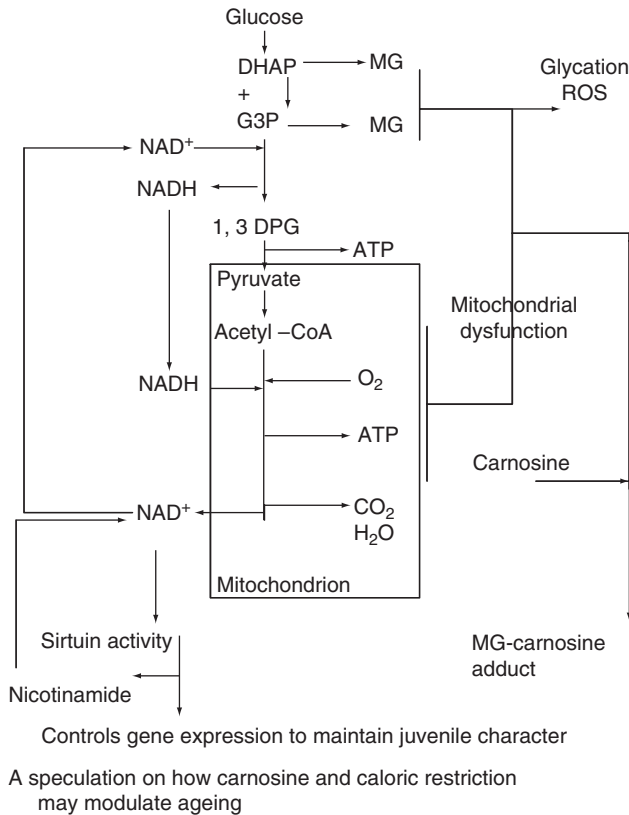
FIGURE 3.2 A speculation on how dietary restriction and carnosine might regulate methylglyoxal (MG) production and consequent generation of altered proteins which characterize the aged state. Increased frequency of glycolysis decreases NAD$^+$ availability and increases likelihood of MG generation and protein modification. Caloric restriction and/or aerobic exercise decreases NAD$^+$ consumption and increase NADH oxidation to facilitate NAD$^+$ regeneration to maintain juvenile character via NAD$^+$-dependent sirtuin activity and decreased MG generation.

evidence suggesting that increased MG production has a causal role in much diabetes-associated pathology (see Ahmed and Thornalley, 2007; and references therein)

The variation in tissue susceptibility to these and other aging-induced changes may partly result from differing levels of those molecules (glutathione, polyamines, carnosine, creatine, pyridoxamine, glyoxalases 1 and 2) which normally exert protective activity against glycoxidating agents such as MG. It is also possible that intermittent glycolysis could be hormetic by upregulating synthesis of some of these defense molecules (Hipkiss, 2007b). Interestingly, carnosine has also been described as a

Methylglyoxal (MG) induced deleterious protein-AGE   **Possible protection by carnosine**

Protein-NH$_2$ + CH$_3$COCHO + Carnosine $\longrightarrow$ Carnosine-MG adduct
(MG)   (excreted?)

↓

Protein-MG adduct

↓

Protein-CO-AGE   + Carnosine $\longrightarrow$ Protein-CO-carnosine adduct
(protein carbonyl)   (carnosinylated-protein)
(inert lipofuscin)
(degraded?)

Protein-NH$_2$ →

↓

Protein-protein–AGE
(cross-linked;
resists proteolysis;
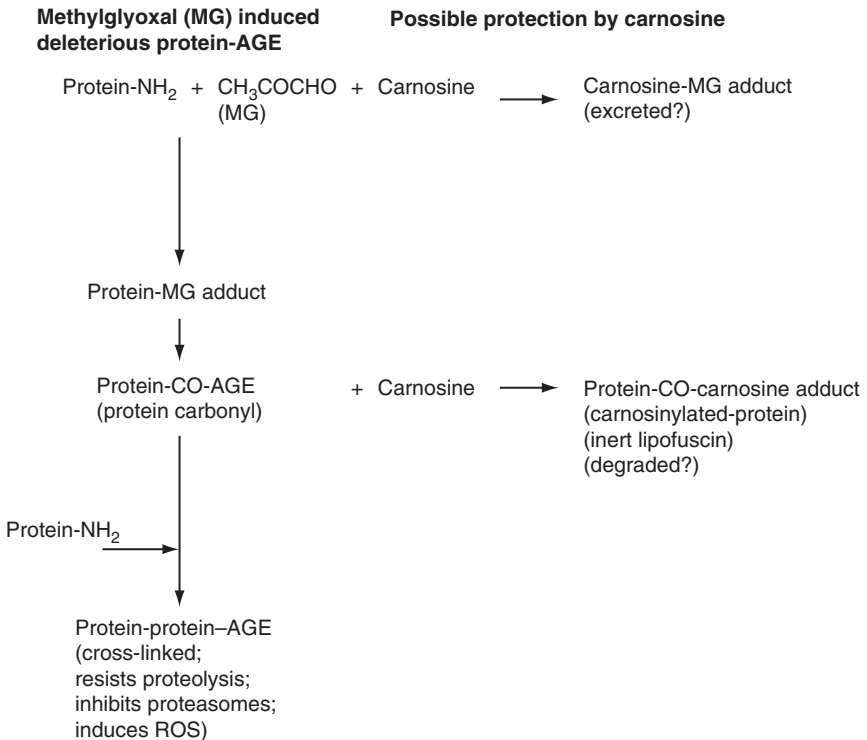inhibits proteasomes;
induces ROS)

FIGURE 3.3 Schematic showing possible sites of intervention by carnosine during formation of cross-linked methylglyoxal-modified proteins. AGE, advanced glycation end-product.

glyoxalase mimetic (Battah *et al.*, 2002), in addition to its other protective activities. Nevertheless, carnosine's ability to protect against MG toxicity may be important physiologically especially with respect to the changes responsible for aging and related pathologies (see Fig. 3.3).

Intracellular carnosine concentration may be subject to metabolic regulation. Destruction of the dipeptide by carnosinase is stimulated by citrate (Vistoli *et al.*, 2006), thus raising the possibility that inhibitory molecules could be created to prevent destruction of the dipeptide in sera. Carnosine's synthesis by carnosine synthetase is downregulated by raised cAMP levels (Schulz *et al.*, 1989), at least in astrocytes. Thus, high glucose concentrations could lower cAMP levels and hence stimulate carnosine synthesis.

It is interesting that many of the studies using model organisms to study how aging is delayed by genetic, physiological, and dietary means have a common feature, that is the upregulation of mitogenesis

(Anderson *et al.*, 2008; Bonawitz *et al.*, 2007; Cunningham *et al.*, 2007; Guarente, 2008; Hipkiss, 2008b; Lopez-Lluch *et al.*, 2008; Rodgers *et al.*, 2008; Soh *et al.*, 2007). This effect is also induced by increased aerobic exercise in mammals where many of the symptoms of aging are suppressed. One possible explanation is that the stimulation of synthesis of mitochondria also increases synthesis of the necessary chaperone proteins which are required for maintenance of protein quality in the growing organelle and the cytosol. Hence, as these proteins also participate in the elimination-altered proteins arising from postsynthetic damage, it is likely that the increased ability to recognize and degrade the aberrant proteins due to erroneous protein synthesis will also improve overall cellular stress-resistance and enhance longevity. Carnosine, when complexed with zinc ions, has been shown to stimulate expression of certain stress proteins, for example, Hsp72 (Odashima *et al.*, 2002, 2006; Mikami *et al.*, 2006; Wada *et al.*, 2006). Furthermore, carnosine may also stimulate synthesis of the stress hormone corticosterone (discussed below) which may in turn upregulate expression of a number of stress proteins. Although very hypothetical these suggestions are relatively easy to test. That the stress protein Hsc70 becomes preferentially glycated in senescent cells and that synthesis of vimentin, another readily glycated protein, is induced by carnosine is perhaps indicative of a relationship between these factors; for example, could upregulation of vimentin synthesis compete with Hsc70 glycation and help delay onset of stress-induced premature senescence?

XVII. CARNOSINE, REGULATION OF PROTEIN SYNTHESIS, AND AGING

It has recently been shown that carnosine can also exert suppressive effects on mRNA translation initiation (Son *et al.*, 2008); the dipeptide inhibited interleukin-8 mRNA translation by suppressing phosphorylation of initiation factor eIF4E in peroxide-activated intestinal epithelial cells and Caco-2 cells. eIF4E Phosphorylation is required for effective mRNA translation, which explains the observed carnosine-mediated decreased synthesis of the proinflammatory cytokine. Carnosine also inhibited phosphorylation of other regulatory proteins ERK1/2 and p38 MAP kinase (Son *et al.*, 2008). This may be important as Davis *et al.* (2005) have shown that accelerated aging can be suppressed by inhibiting p38 MAP kinase phosporylation. The mechanism by which carnosine suppresses kinase phosphorylation is unknown, but it could be a consequence of decreased glycoxidative damage within the cell, due to the presence of carnosine, rather than direct participation of the dipeptide in the signaling pathway.

Defective eIF4E limits mRNA translation initiation and results in life span extension in *C. elegans* since studies show that aging can be delayed by partial inhibition of protein synthesis. Studies in *C. elegans* revealed that senescence was delayed, stress resistance enhanced, and life span extended in eIF4E mutants (Pan *et al.*, 2007; Syntichaki *et al.*, 2007). Similarly, life span of *C. elegans* was extended and stress resistance enhanced when translation was inhibited where synthesis of eleven ribosomal proteins was suppressed using inhibiting-RNAs (RNAi) (Hansen *et al.*, 2007). Certain ribosomal protein defects also have beneficial effects on yeast longevity (Chiocchetti *et al.*, 2007). Explanation of these effects is uncertain (Kaeberlein and Kennedy, 2007), but it is possible that a lower rate of bulk protein synthesis, resulting from decreased translation initiation frequency, also lowers synthesis of error-proteins (Hipkiss, 2007c). This lowered production of biosynthetic aberrant proteins could directly lower the load that the chaperone and proteolytic apparatus must deal with: the chaperone/proteolytic apparatus is responsible for the elimination of altered protein generated postsynthetically as well as those formed by biosynthetic errors. Compared to normal gene products, error-proteins are more readily glycated and oxidatively damaged by ROS (Dukan *et al.*, 2000; Fredriksson *et al.*, 2006) than the normal gene products, thus the mutant organisms would generate fewer protein carbonyls, that normally characterize the senescent state (Stadtman, 1992) than the wild type. Consequently, the decreased level of biosynthetic error-proteins would not only decrease formation of protein carbonyls but also increase the relative availability of chaperone and proteolytic activities for the recognition and elimination of altered proteins arising from deleterious postsynthetic modification (Hipkiss, 2007a).

Interestingly, methionine restriction (40% and 80%) also delays aging in rodents (Miller *et al.*, 2005; Naudi *et al.*, 2007). Methionine is the initiating amino acid in protein biosynthesis; therefore, this could again indicate that decreased translation initiation is an effective antiaging strategy by decreasing biosynthetic formation of error-proteins, similar to the effects of the defective eIF4E initiation factor in nematodes outlined above (Hipkiss, 2008c). It is possible that because carnosine's also has inhibitory effects on eIF4E activity and slows protein synthesis, the beneficial effects on fibroblast senescence and life span could be mediated via a similar mechanism in human cells.

XVIII. CARNOSINE AND CORTICOSTEROIDS

A recent study has shown that intracerebroventricular carnosine administration stimulates corticosterone release in chick brain (Tsuneyoshi *et al.*, 2007). Studies performed some 30–40 years ago showed that

hydrocortisone or cortisone (Cristofalo and Kabakjian, 1975; Macieira-Coelho, 1966) have positive effects on the growth and life span of cultured human fibroblasts. These findings have recently been reactivated where the beneficial effects of glucocorticoids towards cultured human fibroblasts have again been demonstrated (Kletsas *et al.*, 2007). Given that carnosine can also affect fibroblasts life span in a positive manner (McFarland and Holliday, 1994), it is at least conceivable that carnosine's action is mediated via glucocorticoid upregulation. A recent study at the whole animal level has revealed mixed results however, Caro *et al.* (2007) showed that 4 weeks chronic treatment with corticosterone decreased markers of lipid peroxidation but protein glycoxidation and oxidative damage to mitochondrial DNA were both increased in rat liver. Clearly, this is another research area which should be explored.

XIX. CARNOSINE AND AGE-RELATED PATHOLOGY

Accumulation of altered protein forms, particularly protein carbonyl groups, is not only the most common biochemical signature of aging (Levine, 2002) but such aberrant polypeptides are associated with many age-related diseases (Dalle-Donne *et al.*, 2003) as well. As carnosine has the potential to intervene in a number of processes that possibly contribute to the phenomenon we call aging, particularly where generation of altered proteins is involved, it follows that the dipeptide may have some beneficial effects with respect to either the causation or progression of those age-related conditions which also involve accumulation of aberrant protein forms. Table 3.5 lists the possible conditions against which carnosine might exert some therapeutic effects. It should be emphasized that this list is, for the most part, purely speculative and considerable amounts of work needs to carried out to verify or eliminate these suggestions.

XX. CARNOSINE, DIABETES, AND SECONDARY COMPLICATIONS

The secondary complications of diabetes include cardiac and circulatory disorders, peripheral neuropathy, cataractogenesis, and stroke. Over the past decade it has become increasingly evident that much diabetes-associated pathology derives from hyperglycaemia where glucose, or more likely its metabolites and by-products, chemically modify intracellular and extracellular proteins and aminolipids via the process called glycation (Ahmed and Thornalley, 2007; Goh and Cooper, 2008; Magalhaes *et al.*, 2008; Singh *et al.*, 2001; Vlassara and Palace, 2002). The process is termed nonenzymic glycosylation or glycation to distinguish it

TABLE 3.5   Potential age-related conditions against which carnosine could be explored therapeutically

- Diabetes and diabetic complications
- Ischemia
- Neurodegeneration
- Osteoporosis
- Deafness
- Slow wound healing
- High blood pressure
- Heart disease
- Cataractogenesis

from the enzyme-mediated attachment of sugars to proteins or lipids required for proper cell function/distribution. It should be noted that glycation of proteins mediated by glucose is relatively slow, but other common sugars such as galactose or fructose are much more rapid. Furthermore, there is much evidence that certain metabolic intermediates of glucose catabolism (via glycolytic pathway) can almost immediately glycate intracellular and extracellular proteins. As noted above, MG is particularly damaging (Cantero *et al.*, 2007; Desai and Wu, 2008; Dhar *et al.*, 2008; Gomes *et al.*, 2008; Kalapos, 1999; Mirza *et al.*, 2007; Nakayama *et al.*, 2008; Wang *et al.*, 2007; Yander, 2008; Yao *et al.*, 2007) and many studies have suggested that MG is the primary source of much of the deleterious protein glycation which is responsible for diabetic complications (see Rabbani and Thornalley, 2008; Wang *et al.*, 2008 for recent reviews). Hence there has been an extensive search for agents which possess antiglycating activity which may be employed to suppress AGE formation and attendant diabetic complications. It was suggested some time ago that carnosine might be a candidate antiglycating agent for the control of secondary diabetic complications (Hipkiss, 1998; Hipkiss *et al.*, 1995a,b).

Carnosine may help suppress some features of aging at cellular and whole organism levels, possibly by inhibiting the reactivity of ROS and deleterious aldehydes including formation of AGEs (Hipkiss *et al.*, 2002). It is theoretically possible that the dipeptide is beneficial towards those conditions where formation of protein AGEs plays important and most likely causal roles. Even in complication-free diabetics, the levels AGE precursors such as MG and glyoxal are elevated in their sera (Han *et al.*, 2007). So it is likely that carnosine and other carbonyl scavengers might exert beneficial effects towards diabetes and its secondary complications (Hipkiss, 2005). Lee *et al.* (2005) have indeed demonstrated that dietary carnosine suppresses a number of diabetic complications in mice.

There is also evidence indicating that low cellular carnosine levels are associated with diabetes (Gayova *et al.*, 1999; Nagai *et al.*, 2003). A recent study has shown that carnosine can inhibit formation of glycated low-density lipoprotein which normally provokes formation of foam cells associated with circulatory disorders which characterize diabetic complications (Rashid *et al.*, 2007). Furthermore, carnosine can decrease blood pressure in rats (Nagai *et al.*, 2003; Tanida *et al.*, 2005) and possesses vasodilatory activity (Ririe *et al.*, 2000); hypertension is another consequence of diabetes. It has been shown that the toxic effects of fructose and high glucose levels on blood pressure might be mediated via the generation of MG (Wang *et al.*, 2006). MG not only induces peroxynitrite production in vascular smooth muscle cells (Chang *et al.*, 2005) but also plays a causative role hypertension (Wu, 2006). Clearly, if carnosine does indeed scavenge MG *in vivo*, then it could exert protective effects towards hyperglycemia-induced hypertension.

Another complication of diabetes is the loss of peripheral neuronal function. It has recently been shown that carnosine and its zinc complex can ameliorated progressive diabetic neuropathy in mice (Kamel *et al.*, 2008), although the zinc–carnosine complex was more effective than the uncomplexed dipeptide.

An interesting observation is that serum AGE levels are higher in diabetic vegetarians compared to diabetic omnivors (Krajcovicova-Kudlackova *et al.*, 2002). This may be because of the absence of carnosine in vegetarian diets, although a raised intake of fructose by vegetarians is an alternative explanation.

More evidence that carnosine may possess therapeutic potential comes from studies on carnosinase in mice and humans. These studies have shown that higher levels of this enzyme are associated with diabetic end-stage kidney disease in humans, whereas a lower activity seemed to be protective (Freedman *et al.*, 2007; Janssen *et al.*, 2005). A further study using diabetes-prone mice, in which serum carnosine or carnosinase levels were manipulated, showed that the onset of diabetic complications was enhanced when carnosinase activity was increased by transgenic modification with the human carnosinase gene CN1; diabetes was milder and delayed in animals supplemented with carnosine (Sauerhofer *et al.*, 2007).

XXI. CARNOSINE AND NEURODEGENERATION

There is evidence from animal studies that carnosine can affect brain function/activity (Tanida *et al.*, 2007; Thio and Zhang, 2006; Tomonaga *et al.*, 2004, 2005, 2008; Tsuneyoshi *et al.*, 2007, 2008); furthermore, the dipeptide is protective against a number of neurotoxic agents, for

example, *N*-methyl-D-aspartate (NMDA) (Shen *et al.*, 2007a,b), copper (Hornung *et al.*, 2000), zinc (Kawahara *et al.*, 2007), ROS (Kim and Kang, 2007; Kim *et al.*, 2004), and RNS (Calabrese *et al.*, 2005). There is also substantial evidence suggesting that carnosine is protective against ischemia in the brain and induced seizures; both of these are discussed separately below.

Many neurodegenerative diseases are age-related, consequently it is possible that factors which delay general aging could also delay onset of neurodegeneration. Furthermore, many neurodegenerative diseases have at least one feature in common, that is the accumulation of altered proteins, a feature characteristic of aged cells generally. These aberrant protein forms are found as tangles and amyloid plaque in Alzheimer's disease (AD), as Lewy bodies in PD and inclusion bodies in Huntington's disease (HD) (Bossy-Wetzel *et al.*, 2004). From first principles, general explanations for their occurrence are either increased production of altered proteins or their decreased clearance from the tissue. The likelihood that neurodegeneration is accompanied by dysfunction of either the ubiquitin/proteasome system (Paul, 2008) and/or the autophagic apparatus (Bandhyopadhyay and Cuervo, 2007) has been recognized for some time. The possibility that carnosine might stimulate proteolytic activity by, for example, activating proteasome function by increasing nitric oxide synthesis (Thomas *et al.*, 2007), or whether it affects autophagy or chaperone formation/activity, should be explored.

It has long been suggested that ROS may play causal roles in these diseases and in the production of the aberrant protein molecules (de Arriba *et al.*, 2006). In addition, protein damage inflicted by RNS has also been suggested. As discussed above, carnosine has been shown to possess antioxidant activity and also to react with RNS as well, hence the molecule has the potential to be considered as a theraupeutic agent (Calabrese *et al.*, 2008).

There are a number of findings suggesting that agents that facilitate elimination of protein carbonyls (by either proteolytic elimination or by enzymically mediated chemical reduction) may suppress neurodegenerative conditions in model systems (Botella *et al.*, 2004). Consequently, as carnosine may also react with protein carbonyls, it is theoretically possible that it could suppress formation and/the reactivity of protein carbonyls in the brain. Whether carnosine participates in carbonyl reductase activity has not been investigated but it is also a reasonable speculation.

There are numerous examples where carnosine has been demonstrated to be protective activity against neurotoxic agents. For example, the dipeptide was shown to protect the mitochondria of cultured astroglial cells against nitric oxide-induced damage (Calabrese *et al.*, 2005). Carnosine was also shown to protect neurofilament-L against oxidative damage, aggregation, and formation of dityrosine induced by hydrogen

peroxide and cytochrome *c* (Kim and Kang, 2007; Kim *et al.*, 2004). Carnosine was shown to modulate the neurotoxic effects of copper and zinc (Hornung *et al.*, 2000). In the following sections, the possible roles for carnosine as protective agents in specific neurodegenerative conditions are discussed.

XXII. ALZHEIMER'S DISEASE

The causal events in AD are much discussed but increased oxidative/ glycoxidative damage is acknowledged to play a role. One common altered protein form which accompanies AD is a small peptide fragment called amyloid-β-peptide (Aβ-peptide). Aβ-peptide is generated from a larger protein called amyloid precursor protein (APP) via the action of two proteases. It appears that an enzyme (insulin degrading enzyme or IDE) which normally cleaves the Aβ-peptide in the middle declines with age (Caccamo *et al.*, 2005; Farris *et al.*, 2005; Qiu and Folstein, 2006). The reason for IDE's decline in activity is unknown; possibilities include inactivation by ROS, RNS, or glycating agents, decreased gene expression and preferential usage in insulin metabolism.

Glycoxidation events have a role in neurodegenerative disorders, and some recent papers have proposed that MG may be directly involved (Bar *et al.*, 2002; Luth *et al.*, 2005; Munch *et al.*, 1997, 2003; Pamplona *et al.*, 2005, 2008; Reddy *et al.*, 2004; Yan *et al.*, 1994). Other reactive aldehydes such as lipid oxidation products, hydroxynonenal, malondialdehyde, and acrolein are additional sources of protein damage. It is theoretically possible that carnosine or related structures could react with these deleterious aldehydes and thereby suppress their damaging effects towards proteins (Hipkiss, 2007a). It may be significant that a low serum carnosine level has been reported to be associated with AD (Fonteh *et al.*, 2007). Furthermore, a raised level of protein AGEs in cerebral spinal fluid (CSF) (Ahmed *et al.*, 2005; Luth *et al.*, 2005; Shuvaev *et al.*, 2001; Yamagishi *et al.*, 2005) is associated with AD, while homocarnosine levels in CSF generally decline markedly with age (Huang *et al.*, 2005; Janssen *et al.*, 2005). It may also be significant that carnosine is enriched in the olfactory lobe (Barkardjiev, 1997; Bonfanti *et al.*, 1999; Sassoe-Pognetto *et al.*, 1993) and a loss of a sense of smell may be an early symptom of neurodegeneration (Ghanbari *et al.*, 2004; Kovaks, 2004). Given carnosine's homeostatic properties outlined above, it is at least worth considering whether carnosine or homocarnosine possess therapeutic potential toward AD (Hipkiss, 2007a), especially as the choroid plexus possesses a carnosine (homocarnosine) transport protein which may control CSF homocarnosine levels (Teuscher *et al.*, 2004). While carnosine is absent from human CSF, one speculates that homocarnosine might act as an antiglycating agent in CSF (Hipkiss, 2007a). The

age-related decline in CSF homocarnosine levels (Jansen *et al.*, 2006) could at least partially explain the observed increase in glycated proteins CSF of AD patients, as well as the strong relationship between aging and AD.

Many of the proteins which accumulate during neurodegenerative conditions may also become cross-linked by transglutaminase (Andringa *et al.*, 2004; Junn *et al.*, 2003; Karpuj and Steinman, 2004; Selkoe *et al.*, 1982), an enzyme which cross-links the glutamine side chain to a lysine ε-amino group. Transglutaminase protein is also associated with many of the inclusion bodies characteristics of AD, PD, etc. (Junn *et al.*, 2003). It is theoretically possible that carnosine could substitute for the lysine residue ε-amino group in the transglutaminase reaction (Hipkiss, 2007a) to generate γ-glutamyl-carnosine as a hypothetical reaction product. While no such linkage has been reported (or sought) in neuronal tissue from neurodegenerative brain, the predicted γ-glutamyl-β-alanyl-histidine products have been isolated from animal muscle tissues (Kuroda *et al.*, 2000). However, as discussed above, it is possible that such structures might be derived from the spontaneous deamidation of glutamine residues in close proximity to carnosine or from the reaction of the dipeptide with oxidatively induced glutamic semialdehyde.

Carnosine's copper and zinc ion-chelating activity may also contribute to suppression of neurodegenerative conditions (Hipkiss, 2005). Zinc has been reported to be associated with the amyloid which accumulates in AD brain (Bush and Tanzi, 2002; Danscher *et al.*, 1997; Religa *et al.*, 2006), while copper ion-mediated oxidation of neuronal proteins may accompany both AD and PD (Smith *et al.*, 2006). Carnosine has been found to protect cultured neurons against zinc-induced death (Kawahara *et al.*, 2007).

Cell culture studies have shown that carnosine is protective against the toxicity of the Aβ-peptide which accumulates in the AD brain. The dipeptide prevented Aβ-peptide (1–42)-induced glutamate release, but increased expression of the NMDA receptor. It was proposed that carnosine's protective activity was exerted via regulation of glutamate release and independent of the carnosine–histidine–histamine axis (Fu *et al.*, 2008). Similarly, Boldyrev *et al.* (2004c) found that carnosine suppressed the cytotoxicity of Aβ42 in cerebellar granule cells independently of the dipeptides' effects on calcium metabolism and ROS generation. Preston *et al.* (1998) also showed that the toxicity of the Aβ42-related peptide fragment (25–35) towards rat brain endothelial cells was inhibited by carnosine and related structures, although the mechanism involved was not investigated.

There is one explorative study investigating whether there is any correlation between serum carnosinase levels and dementia. The findings, using a small sample size, indicate that while there was no significant

difference between control patients and those suffering from mixed dementia or AD, carnosinase activity was higher in patients who regularly exercised (Balion *et al.*, 2007).

XXIII. PARKINSON'S DISEASE

Parkinson's disease is a neurodegenerative condition associated with the loss of dopaminergic neurons in a region of the brain called the substantia nigra pars compacta. The cause of PD is unknown but it seems that the substantia nigra is particularly susceptible to oxidative damage which in turn induces mitochondrial dysfunction and increased production of ROS, accompanied by the accumulation altered protein species which form aggregates called Lewy bodies. A major component of Lewy bodies is a protein called α-synuclein, an abundant presynaptic protein, but other proteins are present in these structures including ubiquitin, transglutaminase, and a number of heat-shock proteins.

It appears likely that oxidative events including mitochondrial dysfunction play a major role in PD. Among the deleterious agents thought to be involved are peroxynitrite and hydroxyl radicals (Yokoyama *et al.*, 2008). As noted above, carnosine has been shown to inhibit protein damage mediated by peroxynitrite and hydroxyl radicals in astroglial cells (Nicoletti *et al.*, 2007). There is some evidence that carnosine can suppress some of the oxidative damage associated with PD using a model system, and possibly inhibit fibrillization of α-synuclein (Herrera *et al.*, 2008).

In order to investigate PD in animal models, one approach is to use a chemical called 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a known neurotoxin which induces symptoms similar to PD in animals and humans. When this compound was injected into senescence-accelerated mice (SAMP1), the animals demonstrated short-term tremor, weight loss and pronounced rigidity. Changes in the brain were observed including increased levels of protein carbonyls, lipid hydroperoxides, and monoamine oxidase-B activity. However, if these MPTP-treated animals were also treated with carnosine (100 mg/kg) for 14 days, the weight loss and rigidity were decreased, as were the levels of protein carbonyls, lipid hydroperoxides, and monoamine oxidase-B activity in their brains (Boldyrev *et al.*, 2004a,b,c). These observations seem to suggest that carnosine suppresses some of PD-like changes induced by MPTP. At present it is unknown whether carnosine is similarly beneficial in humans.

It has recently been shown that oxidative damage to glyceraldehyde dehydrogenase (GDH), an important glycolytic enzyme, occurs in the frontal cortex in PD patients (Gomez and Ferrer, 2009). Not only would this limit ATP synthesis and generation of many necessary metabolic

intermediates, but would also increase formation of MG. As noted above, MG is a highly toxic agent which rapidly reacts with available protein and lipid amino groups, and can provoke mitochondrial dysfunction and can induce many of the biochemical symptoms of aging. As also noted above, carnosine has been shown to protect proteins against MG-induced modification.

α-Synuclein is a major component of the Lewy bodies which accompany PD. There is an extensive literature about the roles of α-synuclein mutations and its metabolism, modification, and modes of aggregation (Bisaglia *et al.*, 2009). There is some evidence that carnosine can inhibit α-synuclein oligomerization in a model system (Kang and Kim, 2003; Kim *et al.*, 2002). Another paper has shown that an early event in Lewy body diseases is formation of adducts between α-synuclein lysine amino groups and malondialdehyde (MDA), a lipid peroxidation product, in the substantia nigra and frontal cortex (Dalfo and Ferrer, 2008). Many years ago it was shown that carnosine inhibited both MDA-mediated toxicity in cultured neuronal cells and formation of protein carbonyls and protein cross-linking (Hipkiss *et al.*, 1997).

It appears that the α-synuclein contains intramolecular cross-links possibly mediated by tissue transglutaminase (Andringa *et al.*, 2004; Muma, 2007; Ruan and Johnson, 2007). As noted above there is a theoretical possibility that carnosine may be a competitive inhibitor for tissue transglutaminase which could prevent formation of the α-synuclein cross-links which may prevent Lewy body formation and proteasome inhibition.

There is evidence that proteasomal function is compromised in PD (McNaught *et al.*, 2003), which could promote accumulation of α-synuclein, etc. and consequent inclusion body formation. Mutations in the genes for at least two proteins, Parkin and ubiquitin carboxyterminal hydrolase L1, which are components of the ubiquitin–proteasome system (Pallancke and Greenamyre, 2006) are associated with PD. Additionally, mutations in the gene for a protein, termed Pink1, which may have a role in mitochondrial function and appears to associate functionally with Parkin (Clark *et al.*, 2006), are also associated with PD. One possibility is that Pink1 regulates a mitochondrial protease HtrA2 also called Omi (Plun-Favreau *et al.*, 2007) which may be involved in protein quality control. Whether stimulation of the proteasomal system and/or chaperone-mediated autophagy by carnosine-induced increased stress protein synthesis is beneficial to the compromised proteolytic apparatus is unknown.

A recently proposed explanation of PD involves the formation of adducts between dopamine and the products of the peroxidation of arachidonic acid and docosahexanoic acid (Liu *et al.*, 2008b). At least two compounds, hexanoyl-dopamine and propanoyl-dopamine, derived

from arachidonic acid and docosahexanoic acids respectively, are cyto-toxic to cultured neuronal cells via ROS production and mitochondrial dysfunction. It is conceivable that carnosine could substitute for dopa-mine as the dipeptide does exhibit antioxidant activity. This suggestion predicts that hexanoyl-carnosine and propanoyl-carnosine might be gen-erated. Interestingly it has recently been reported that dietary supplemen-tation of young pigs with docosahexanoic acid provokes a decrease in muscle carnosine levels (Li *et al.*, 2008), which may indicate some sort of relationship between the dipeptide and unsaturated fatty acids and is consistent with the idea that carnosine and docosahexanoic acid peroxi-dation products form adducts together.

Although there is no cure for PD, a common treatment to maintain dopamine supply, and hopefully slow down the degeneration, involves using L-dopa as a source of dopamine. It has been reported, however, that some of the monoamine oxidase-generated oxidative metabolites of L-dopa are neurotoxic, most likely due to the generation of aldehyde groups on them (Burke *et al.*, 2004). Given carnosine's avidity for a variety of metabolic aldehydes (discussed above), it is theoretically possible that the dipeptide could react with 3,4-dihydroxyphenylglycolaldehyde (dopal), the product of monomine oxidase activity on L-dopa, especially as L-dopa treatment in rats elevates dopal levels in their brains 18-fold (Fornai *et al.*, 2000).

Combining carnosine treatment with L-dopa therapy in PD subjects has recently been examined in a study using 36 patients (Boldyrev *et al.*, 2008). It was found that carnosine treatment (1.5 g/day) significantly improved a number of neurological symptoms including decreased rigid-ity of the hands and legs, and increased hand movement and leg agility. At a biochemical level, it was found that the level of protein carbonyls in blood plasma was decreased after 30 days carnosine treatment; further-more, the levels of red cell Cu/Zn-superoxide dismutase were increased in the carnosine-treated PD patients. While these results are very encour-aging with respect to the efficacy of carnosine with respect to PD and possible other neurological conditions involving aldehydes and ROS species, they require much larger and extensive trials to confirm these findings. Nevertheless, they do seem to indicate that carnosine can exert some therapeutic benefit despite the presence of serum carnosinases.

There has been another study where carnosine has been employed in combination with a source of dopamine. Sozio *et al.* (2008) chemically linked the β-amino group of carnosine to an L-dopa precursor and then measured the release of dopamine over a 12-h period in rats. It was found that when presented as a co-drug the level of tissue dopamine was retained at higher levels compared to when the animals were given free L-dopa. There were no major effects of Parkinsonian behavior noted,

although given the very brief time over which these experiment was carried out, this is not unexpected.

XXIV. CARNOSINE AND ISCHEMIA

Evidence that carnosine possesses anti-ischemic activity emerged from Russian studies some years ago (see Stvolinsky and Dobrota, 2000 and references therein). Since then even more encouraging evidence has been obtained; the dipeptide has therapeutic potential against a number of ischemic conditions in brain, liver, heart, and kidney. Studies of brain ischemia or strokes using animal models (Gallant *et al.*, 2000; Rajanikant *et al.*, 2007; Tang *et al.*, 2007; Yasuhara *et al.*, 2008) have shown that carnosine is protective, even when added *after* the ischemic injury (Dobrota *et al.*, 2005; Rajanikant *et al.*, 2007; Tang *et al.*, 2007). As part of the possible explanations for its protective action, it has been suggested that carnosine's ability to scavenge the lipid peroxidation products hydroxynonenal (Tang *et al.*, 2007) and malondialdehyde (Dobrota *et al.*, 2005) help to compensate for any ischemia-induced deficit in antioxidant activity. It has also been shown in the postischemic mouse brain that carnosine treatment, 2 h *following* the experimental stroke, caused a decrease in ROS levels and matrix metalloproteinase protein levels and activity, whereas glutathione levels were preserved (Rajanikant *et al.*, 2007). It appeared that carnosine treatment 2 h *following* the experimental stroke was also effective in decreasing infarct area, but when added after 4 h carnosine was ineffective, indicating a therapeutic window if carnosine is to be considered for treatment of strokes in humans. Interestingly, a recent study has shown that the presence of bestatin, an inhibitor of the enzyme carnosinase which cleaves the dipeptide into its constituent amino acids, histidine, and β-alanine, suppressed the efficacy of carnosine in the mouse brain stroke model (Min *et al.*, 2008). Indeed the presence of bestatin increased stroke severity but did not raise cerebral carnosine levels. This may indicate that conversion of the dipeptide into histidine and β-alanine is required for efficacy; alternatively bestatin may have been exerting other unidentified effects. The carnosine analogues anserine and *N*-acetyl-carnosine were much less effective than carnosine in decreasing stroke infarct size. Homocarnosine, however, does protect cultured neuronal cells against ischemia (Tabakman *et al.*, 2004).

   Protective effects have also been observed against ischemia in liver (Fouad *et al.*, 2007), heart (Alabovsky *et al.*, 1997), and kidney (Fujii *et al.*, 2005, 2003; Kurata *et al.*, 2006). Despite these clear observations of efficacy, the underlying mechanisms responsible for carnosine's effects remain uncertain but presumably include its antioxidant and carbonyl-scavenging

activities and possible actions on matrix metalloproteinases (Rajanikant *et al.*, 2007) and histamine receptors (Kurata *et al.*, 2006).

XXV. CARNOSINE AND OSTEOPOROSIS

It is possible that regulation of protein glycation, and formation of protein AGEs, can affect osteoporosis (Hein, 2006). Some recent studies suggest that glycation can affect bone's mechanical property (Shiraki *et al.*, 2008; Tang *et al.*, 2009) possibly by provoking deleterious changes in osteoblast function (Franke *et al.*, 2007). Whether dietary carnosine would affect glycation of bone proteins is an obvious question which has been addressed by a Japanese group who have produced evidence suggesting the carnosine–zinc complexes are therapeutic in terms of bone loss in animal models and humans (Kishi *et al.*, 1994; Sugiyama *et al.*, 2000; Yamaguchi and Kishi, 1993; Yamaguchi and Matsui, 1996). It is suggested that the carnosine–zinc complex both stimulates bone formation by osteoblasts and decreases bone resorption by the osteoclasts (Yamaguchi, 1995; Yamaguchi and Kishi, 1995a). The mechanisms involved remain obscure, but it appears that in cultured mouse marrow cells the carnosine–zinc complex inhibits osteoclast cell formation, when present at between 10^{-6} and 10^{-4} M, by inhibiting the action of transforming growth factor-β (Yamaguchi and Kishi, 1995a) and parathyroid hormone, possibly by interfering with calcium signaling (Yamaguchi and Kishi, 1995b). It is also possible that the zinc–carnosine complex enhances the anabolic effects of estrogen on osteoblasts (Yamaguchi and Matsui, 1997). It is clear from these observations that carnosine, when complexed with zinc, may have beneficial effects towards control of osteoporosis but many more studies, including double-blind trials in humans, are required before any unequivocal statement of its efficacy can be made.

XXVI. CARNOSINE AND CATARACTOGENESIS

A Russian group headed by Barbizhayev have produced a substantial body of work emphasizing carnosine's potential for the treatment of lenticluar cataracts in humans (Barbizhayev, 2008; Barbizhayev *et al.*, 2004). In particular, Barbizhayev has suggested (Barbizhayev *et al.*, 2001) that *N*-acetylcarnosine, which is unsusceptible to the action of serum carnosinase, might be useful as a prodrug as the acetyl group is apparently readily cleaved intracellularly to release carnosine which then exerts its anticataractogenic effects, most probably via a combination of antioxidant and antiglycating activities. It is thought that there is little carnosinase activity in the eye lens. The use eyedrops containing

N-acetyl-carnosine over trial periods of 2 and 6 months were reported to alleviate vision deficiency (lens opacity, visual acuity) associated with cataractogenesis, compared to placebo group. The improvements were sustained for 24 months (Barbizhayev, 2004, 2005).

XXVII. CARNOSINE AND DEAFNESS

Production of ROS is associated with deafness in animals and humans. It appears that carnosine can suppress loss of hearing induced by antibiotics and other agents, although it is uncertain as to the precise mechanisms involved (Zhuravskii *et al.*, 2004a,b). Early studies had shown, however, that carnosine exhibited excitatory activity to the afferent fibers in the lateral line organ of frogs (Mroz and Sewell, 1989; Panzanelli *et al.*, 1994) which may indicate an evolutionary role of the dipeptide in sound detection.

XXVIII. CARNOSINE AND CANCER

Antineoplastic activity of carnosine was first reported more than two decades ago (Nagai and Suda, 1986). L-Carnosine's ability to kill cultured transformed cells (3T3 cells and HeLa cells), selectively, was found to be dependent on the absence of pyruvate in the growth medium (Holliday and McFarland, 1996, 2000); D-carnosine was nontoxic to HeLa cells. When pyruvate or certain other metabolic intermediates (oxaloacetate and α-ketoglutarate) were present in the growth medium, the toxic effects of carnosine towards the transformed cells were inhibited, but citrate, isocitrate, succinate, fumarate, and malate had no effects upon carnosine's ability to kill the cells. The explanation of carnosine's toxicity towards transformed cells is very uncertain. It is possible that carnosine may be inhibiting glycolysis by reacting with glyceraldhyde-3-phosphate, and thereby limiting the supply of metabolic precursors and possibly ATP, whereas the addition of pyruvate, oxaloacetate, and α-ketoglutarate enables these limitations to be overcome. It should be pointed out that many transformed cells are highly dependent on glycolysis for their ATP supply and are more sensitive to agents that interfere with this pathway.

The presence of dietary carnosine in vitamin E-deficient rats was found to increase mammary tumor latency, while not affecting tumor incidence (Boissoneault *et al.*, 1998). Another beneficial effect of carnosine in relation to cancer has recently been reported: carnosine was shown to inhibit metastasis of hepatocarcinoma SK-Hep-1 cells (Chung and Hu, 2008). Unlike the effects reported above, carnosine did not affect the viability of these cells but instead the dipeptide inhibited cell migration and invasion. The mechanism responsible apparently involves a decrease

in extracellular matrix metalloproteinase-9 (MMP-9), an activity necessary for tumor invasion and angiogenesis. However, carnosine did not directly affect MMP-9 activity. Instead the dipeptide appears upregulate expression of the antimetastatic gene nm23-H1, whose gene product inhibits MMP-9 gene expression, and thereby suppresses synthesis of this activity necessary for tumor invasion and metastasis.

XXIX. CARNOSINE AND WOUND HEALING

One problematic aspect of the aged organism is slower wound healing. There is evidence that carnosine can have beneficial effects here (Roberts *et al.*, 1998). When carnosine is complexed with zinc to form ''polaprezinc,'' it behaves as an antiulcer drug which also possesses wound-healing activity (Nagai *et al.*, 1986). Investigation of the possible mechanisms involved has revealed that the zinc–carnosine complex may stimulate synthesis of insulin-like growth factor-1 (Watanabe *et al.*, 1998) and decreased secretion of interleukin-8 (IL-8), due to suppression of IL-8 mRNA expression in gastric epithelial cells. Polaprezinc also downregulated NF-κB activation by a number of activators suggesting overall anti-inflammatory action (Shimada *et al.*, 1999), but did not involve modification of prostaglandin E_2 production in gastric epithelial cells (Arakawa *et al.*, 1990). Further investigation revealed that zinc–carnosine induced expression of the stress protein Hsp72 while inhibiting NF-κB activation in colonic mucosa (Odashima *et al.*, 2002, 2006). These observations may help explain the beneficial effects of polaprezinc in rodent and human gut (Mahmood *et al.*, 2007). Polaprezinc also ameliorated aspirin-induced mucosal injury in rats (Naito *et al.*, 2001) most probably by inhibiting the increase in neutrophil myeloperoxidase via inhibition of TNF-α expression.

Vimentin is thought to play a role in wound healing (Mor-Vaknin *et al.*, 2003), and carnosine has been shown to stimulate vimentin expression in rat fibroblasts (Ikeda *et al.*, 1999). Therefore, it is possible that this provides an additional mechanism for carnosine's beneficial effects on wound healing.

XXX. CARNOSINE AND IMMUNE FUNCTION

There is some evidence suggesting that carnosine can upregulate immune function. Carnosine's ability to react with hypochlorite anions (Formazyuk *et al.*, 1992; Quinn *et al.*, 1992) generated in activated leukocytes via the myeloperoxidase reaction, suggests that the dipeptide may limit hypochlorite-mediated oxidation *in vivo* (Pattison and Davies, 2006)

and moderate neutrophil function (Tan and Candish, 1998). There is also some evidence that carnosine can suppress contact hypersensitivity in mice, but the mechanisms involved have not been studied in detail (Reeve *et al.*, 1993a,b).

XXXI. CARNOSINE, CALCIUM, AND HEART FAILURE

Carnosine occurs in cardiac muscle at concentrations between 2 and 10 mM (Roberts and Zaloga, 2000). Heart failure is thought to be associated with dysregulation of myocardial calcium metabolism resulting in contractile failure. There is evidence that carnosine can improve cardiac contractility, possibly via its effects on regulation of intracellular calcium levels, in a concentration-dependent manner (Zaloga *et al.*, 1996). Studies in rats have shown that carnosine increases the levels of free calcium ions while also increasing the sensitivity of the contractile proteins to calcium (Batrukova and Rubstov, 1997; Roberts and Zaloga, 2000; Zaloga *et al.*, 1997). It is unknown if there is any relationship between heart failure and myocardial carnosine levels in humans patients, although it is known that tissue carnosine levels are decreased in animals suffering from trauma and chronic infection which are associated with impaired cardiac contractility (Roberts and Zaloga, 2000).

XXXII. CARNOSINE AND AUTISTIC SPECTRUM DISORDERS

Autism and Asperger's syndrome are regarded as pervasive developmental disorders. Autism is a neurological disorder associated with impairment of language, cognition, and socialization, whereas Asperger's syndrome is an autistic condition not associated with language delay or intellectual impairment. The causes of these conditions are unknown. In a double-blind, placebo-controlled, trial it was found that that carnosine supplementation improved the behavior, communication, and socialization in children with autistic spectrum disorders (Chez *et al.*, 2002). The mechanism responsible for these effects is very uncertain, but it has been hypothesized that increased oxidative stress may be associated with autism (Chauhan and Chauhan, 2006; Chauhan *et al.*, 2004; Yorbik *et al.*, 2002), and that polymorphisms in the gene coding for the aldehyde-scavenging enzyme glyoxalase 1 could be a susceptibility factor (Junaid *et al.*, 2004). Other workers have questioned this conclusion (Rehnstrom *et al.*, 2008; Sacco *et al.*, 2007; Wu *et al.*, 2008). However, a recent finding by Fujimoto et al (2008) has shown that expression of glyoxalase mRNA in white blood cells correlated inversely with the onset of depression in bipolar disorder patients, compared to controls. It is possible that changes

in glyoxalase expression in neocortex tissue may play a role in autism (Sacco *et al.*, 2007) as increased protein AGEs have been detected in postmortem autistic brain (Junaid *et al.*, 2004). These observations may underline carnosine's effects on the autistic children as the dipeptide possesses antioxidant activity and is protective against methylglyoxal-mediated protein modification. However, it should be emphasized that there have been no other published reports of the beneficial effects of carnosine towards autistic spectrum disorders.

XXXIII. CARNOSINE AND BLOOD PRESSURE

There is evidence that carnosine is a vasodilator (Ririe *et al.*, 2000) and thus can lower blood pressure (Niijima *et al.*, 2002; Tanida *et al.*, 2005). It has been shown that carnosine promotes synthesis of nitric oxide (Nicoletti *et al.*, 2007; Tomonaga *et al.*, 2005), a well-known dilator of blood vessel walls. Also carnosine can inhibit angiotensin-converting enzyme (ACE) activity (Hou *et al.*, 2003; Nakagawa *et al.*, 2006) possibly via effects on cGMP and nitric oxide, which again points to the possibility that the dipeptide or carnosine-enriched foods could be explored to combat raised blood pressure in humans.

XXXIV. CARNOSINE AND CONSUMPTION OF ALCOHOLIC BEVERAGES

Consumption of alcoholic drinks leads to the generation of acetaldehyde in the tissues, predominantly the liver but also in the brain. Acetaldehyde can react with protein amino groups to generate carbonyls with the potential for cross-linking to other macromolecules. It is thought that acetaldehyde generation is a major source of "hangovers" experienced following excessive alcohol consumption. Given carnosine's ability to react with acetaldehyde and protect cultured human fibroblasts and lymphocytes against its toxicity (Hipkiss *et al.*, 1998a) as well as prevent cross-linking between protein and DNA (Hipkiss *et al.*, 1995b), it has been suggested that ingestion of carnosine, either as a supplement or as a high-carnosine food (meat), could be an effective way to prevent "hangovers" (Hipkiss, 1998), as well as protecting the brain and other tissues against alcohol-induced glycoxidative damage. This should be relative easy to test.

Beneficial effects of carnosine have been described with respect to ethanol-induced liver injury in mice (Liu *et al.*, 2008a,b). It was found that following 3 weeks of ethanol treatment (present in drinking water), subsequent exposure to carnosine decreased liver malondialdehyde

levels by around 40% compared to ethanol-treated animals. The dipeptide also promoted a decline in indices of cell damage (release of liver enzymes), increased glutathione content and catalase and glutathione peroxidase activities, and downregulated expression of inflammatory-associated cytokines (IL-6 and TNF-α).

XXXV. CARNOSINE AND HIGH FRUCTOSE FOODS AND DRINKS

There has been much interest in the metabolic effects of fructose and whether its consumption should be restricted due to the sugar's potential deleterious effects with respect to diabetes-associated phenomena (Abdel-Sayred et al., 2008; Brown et al., 2008; Le and Tappy, 2006; Miller and Adeli, 2008). In particular, fructose glycates proteins far more readily glucose to generate protein AGEs, and as a consequence, could possibly be responsible for increasing the incidence of type-2 diabetic complications (Jia and Wu, 2007; Lo et al., 2008). Additionally, fructose is also a ready metabolic source of methylglyoxal which, as already described, is a highly deleterious agent which is thought to be a major causal agent of AGE formation and therefore much of the secondary complications of type-2 diabetes. As carnosine can react with methylglyoxal and fructose to prevent their damaging effects on proteins at the test-tube level, one might consider whether increasing tissue carnosine levels might be beneficial in high fructose diets. However, no such study has yet been carried out to test whether any of these ideas are justified. A further observation has recently been revealed in that high fructose consumption by men can increase the risk of gout due to an increased production of uric acid (Choi and Curhan, 2008; Gao et al., 2008). Consequently it would be interesting to determine whether carnosine ameliorates uric acid production in humans.

XXXVI. CARNOSINE AND DIALYSIS FLUIDS

Treatment of kidney failure involves dialysis using heat-sterilized dialysis fluids. Because the dialysis fluid contains glucose, the heating inevitably generates glucose degradation products such as methylglyoxal, glyoxal, and acetaldehyde, which are well recognized for their ability to induce AGEs on protein targets. Hence dialysis with aldehyde-containing dialysis fluid will not be expected to improve kidney health, but exacerbate the kidney dysfunction. Because of carnosine's ability to protect proteins against aldehydic glycating agents, the possibility that the dipeptide may decrease reactivity of the AGE precursors was explored

(Alhamdani *et al.*, 2007a,b). It was shown that heat-treated peritoneal dialysis fluid compromised the viability of cultured human peritoneal mesothelial cells, whereas the additional presence of carnosine in the incubation medium considerably enhanced cell viability, and markedly decreased cell-associated protein carbonyl groups and ROS generation. These observations obviously suggest that carnosine could be employed to either remove deleterious glucose degradation products from dialysis fluid prior to use, or that the dipeptide could be added to dialysis fluid to suppress the reactivity of the protein damaging agents. However, in the latter case it is uncertain whether patients' kidneys would be able to deal with (i.e., selectively excrete) the putative aldehyde–carnosine adducts.

XXXVII. POSSIBLE WAYS TO INCREASE TISSUE CARNOSINE LEVELS: PHYSIOLOGICAL REGULATION

There have been relatively few studies of age-related changes in tissue carnosine levels despite the fact that the initial observation of the dipeptide's ability to suppress some features of senescence were made more than 15 years ago. Carnosine levels have been reported to decline with age in the rats (Johnson and Hammer, 1992; Stuerenburg and Kunze, 1999) and human muscle (Stuerenburg and Kunze, 1999). More recently, Tallon *et al.* (2007) found evidence of carnosine's age-related decline in human muscle fibers.

On the assumption that increasing tissue levels of carnosine might be beneficial in terms of aging and some of its related conditions, this can be achieved either by physiological regulation or by dietary supplementation. Muscle carnosine levels are generally higher when accompanied by intense exercise in fast-twitch type II fibers compared to slow-twitch type I fibers. It has been found that muscle levels of the dipeptide can be increased following resistance exercise in humans (Hill *et al.*, 2006) and there are also reports that very high levels of carnosine are present in highly trained race-horses (Harris *et al.*, 1990). In humans, the carnosine content of vastus lateralis muscle is generally high in sprinters and body builders (Tallon *et al.*, 2005); 8 weeks intensive training resulted in a doubling of the carnosine content of the vastus lateralis muscle (Kim *et al.*, 2005).

In athletes involved in explosive/intense muscle exercise, it is likely that the raised carnosine levels are required as physiological buffers. Hence it is possible that raising carnosine levels may improve muscle performance by increasing buffer capacity. In an attempt to increase carnosine levels by dietary means, but circumventing the effects of serum carnosinase, increasing β-alanine intake has been investigated

(Harris *et al.*, 2006). Studies have shown that the availability of β-alanine may limit carnosine synthesis, histidine being generally available metabolically. Therefore, it was suggested that dietary supplementation with β-alanine could raise carnosine synthesis in the tissues and studies on human subjects have shown that β-alanine is effective in raising muscle carnosine levels (Harris *et al*, 2006). Furthermore, β-alanine supplementation for 4 and 10 weeks increased vastus lateralis carnosine content by 58% and 80%, respectively, in subjects subjected to high-intensity cycling. A similar study using Vietnamese sports-science students showed that β-alanine supplementation promoted an increase in muscle carnosine concentration (Kendrick *et al.*, 2008), although there were no improvements in any of the exercise parameters measured. In a double-blind randomized study of 26 elderly subjects, aged between 55 and 92 years, it was found that β-alanine supplementation for 90 days improved muscle endurance (physical working capacity) by 28%, presumably due to the increased synthesis of carnosine (Stout *et al.*, 2008). It is interesting that while no beneficial effects in terms of muscle performance were observed in young subjects (Kendrick *et al.*, 2008), whereas in the elderly, improvement was detected (Stout *et al.*, 2008), presumably due to the lower tissue carnosine levels which limit performance in old muscles.

It should be pointed out that subjects consuming β-alanine as a supplement (40 mg/kg body weight) experienced symptoms of flushing, skin irritation and prickly sensations for up to 1 h, first of the ears, forehead and scalp and then the trunk, arms, hands, spine, and buttocks. Lowering the β-alanine dose to 10 mg/kg body weight effectively eliminated these symptoms (Harris *et al.*, 2006). Interestingly, consumption of chicken broth (enriched in carnosine) containing the equivalent of 40 mg/kg body weight β-alanine did not induce any of the unpleasant symptoms, but carnosine was not detected in the plasma of these subjects.

Whether it is possible to raise carnosine levels in human brain is unknown. One study in rats has shown that oral administration of chicken extract (a major source of carnosine in humans too) did provoke an increase in brain carnosine levels: a single dose of the chicken extract led to an increase in carnosine levels within 30 min in plasma, but 1 or 2 h duration were required for increased levels of carnosine to be observed in the cerebral cortex, hypthalamus, and hippocampus (Tomonaga *et al.*, 2007). It is uncertain whether these effects result from direct uptake of the carnosine from plasma or a consequence of de novo synthesis. In a study using senescence-accelerated mice (SAMP8), it was found that oral supplementation with creatine provoked, at 25 weeks of age, a transient 88% increase in muscle carnosine content, accompanied by a 40% increase in anserine content, which coincided with an improvement in resistance to contractile fatigue (Derave *et al.*, 2008). At 60 weeks, no differences were detectable between the creatine-supplemented and control animals in terms of their muscle

carnosine and anserine levels. The mechanism responsible for this effect is uncertain but could involve an upregulation of carnosine synthesis or its decreased catabolism, but why either of these is influenced by creatine supply remains unclear. This study also showed that muscle carnosine declines by 45% with age (from 10 to 60 weeks) in the control SAMP8 mice.

Relatively little is known of the factors that control carnosine synthesis, although as mentioned above, the enzyme involved, carnosine synthetase, does appear to be regulated by cyclic AMP (Schulz *et al.*, 1989) which suggests the possibility that conditions which lower cyclic AMP levels may increase carnosine synthesis. Thus, increased glucose metabolism via glycolysis might be accompanied by an upregulation of carnosine synthesis. It is possible that this suggested relationship is beneficial due to carnosine's ability to suppress the reactivity of a major deleterious byproduct of the glycolytic pathway, methylglyoxal (MG) (discussed above). The primary enzyme responsible for carnosine's hydrolysis into β-alanine and histidine is carnosinase, an activity which is stimulated by citrate (Vistoli *et al.*, 2006). These observations raise the possibility that intracellular carnosine levels may be subject to metabolic regulation due to the effects of major metabolic intermediates and effectors on the enzymes responsible for its synthesis and degradation.

Carnosine levels in the tissues have been seen to decline following trauma and during chronic infection (Fitzpatrick *et al.*, 1980). It is interesting that pathological states are associated with decreased cardiac function possible due to problems with cardiac muscle contraction. It has been suggested that decreased carnosine levels may play a role in decreased muscle contractivity in a number of disease states including congestive heart failure (Roberts and Zaloga, 2000).

XXXVIII. POSSIBLE WAYS TO INCREASE TISSUE CARNOSINE LEVELS: DIETARY SUPPLEMENTATION

While carnosine is absorbed intact from the gut, the presence of serum carnosinase is frequently cited as an impediment to the dipeptide's potential efficacy. However, studies have shown that serum carnosine levels are raised at least temporarily, up to 4–5 h, following a carnosine-containing meal (Antonini *et al.*, 2002; Park *et al.*, 2005). Such studies indicate a window of opportunity for carnosine administration. One approach to overcoming the carnosinase effect would be to employ a carnosinase inhibitor such as bestatin, although undoubtedly there would be some side effects. The fact that carnosinase has been shown to be upregulated by citrate (Vistoli *et al.*, 2006) may permit the design of specific inhibitory molecules. Another approach would be to employ a form of carnosine which is resistant to carnosinase attack such as

N-acetyl-carnosine or the decarboxylated form carcinine. In fact *N*-acetyl-carnosine has been proposed as a prodrug to treat cataracts in the eye lens, as the acetyl group is readily removed intracellularly (Barbizhayev *et al.*, 2004) (see section on cataracts for more details). An alternative way to evade serum carnosinase activity would be to introduce carnosine via nasal administration (Hipkiss, 2005). This route may be particularly appropriate for raising carnosine levels in the brain as the olfactory lobe is normally enriched in the dipeptide.

Synthesis of carnosine analogues resistant to carnosinase attack is another method which has been employed in an attempt to circumvent the problem of serum carnosinase (Bellia *et al.*, 2008; Cacciatore *et al.*, 2005; Calcagni *et al.*, 1999; Guiotto *et al.*, 2005b). Some of the resultant structures with the β-alanine replaced by 2,3-diaminoproprionic acid residue and with acetyl groups on either of its amino groups were shown to not only resist attack by carnosinase but also inhibit the enzyme's ability to cleave carnosine, while still retaining hydroxyl radical-scavenging activity and preventing peroxynitrite-mediated tyrosine nitration (Cacciatore *et al.*, 2005). Other structures synthesized have included sulfonamido-pseudopeptides, tauryl-histidine, tauryl-1-methylhistidine, and tauryl-3-methylhistidine (Calcagni *et al.*, 1999), while another Italian laboratory generated cyclodetrin conjugates of carnosine, attached via the dipeptide's β-amino group to either carbon-3 or carbon-6 of the glucose moiety. These glycosidic derivatives were resistant to carnosinase attack, and seemed to be better inhibitors of copper-induced lipid peroxidation than the parent dipeptides, carnosine, and anserine (Bellia *et al.*, 2008). There were no reports on the efficacy of the cyclodextrin conjugates with respect to carnosine's antiglycating activity, however.

XXXIX. IS THERE ANY EVIDENCE THAT CHANGES IN DIETARY CARNOSINE HAVE ANY EFFECTS IN HUMANS?

There have been few studies on carnosine consumption in humans. Due to the documented presence of carnosinase in blood, many scientists have assumed that the dipeptide' survival would be relatively short due to its rapid hydrolysis by the enzyme. Nevertheless, a study by Gardner *et al.* (1991) showed that plasma carnosine levels peaked at over 180 mg/ml, 0.5 h after intake of a beverage containing 3 g of carnosine. Maximal carnosine levels in urine occurred within 2 h. In another study, Park *et al.* (2005) showed that ingestion of cooked ground beef containing 248 mg of carnosine led to plasma carnosine concentration rising from essentially 0 to around 30 mg/ml within 3.5 h and thereafter rapidly declining such that none was detectable 2 h later. These studies indicate

that, despite the presence of serum carnosinase, ingestion of carnosine can lead to raised levels of the dipeptide in blood which could then lead to effects on tissue carnosine.

Studies of the effects of carnosine consumption on humans have been rare. One study showed that, following intake of a bolus of carnosine (450 mg), serum total antioxidant activity was increased by 11% 1 h after ingestion (Antonini et al., 2002). Such an increase in antioxidant function is consistent with the dipeptide's recognized antioxidant activity. It is possible that human brain function can be affected by dietary carnosine as it has been shown that the dipeptide (two 400 mg doses per day) can modulate the behavior, socialization, and communication skills of autistic children (Chez et al., 2002 and discussed above). Homocarnosine, which also possesses anticonvulsant activity in humans, is found in human CSF. It is unknown whether the sevenfold age-related decline in homocarnsine levels in human CSF (Huang et al., 2005; Janssen et al., 2005) contributes to the onset or progression of any age-related pathology. However, as protein AGEs accumulate in CSF of AD patients (Ahmed et al., 2005; Shuvaev et al., 2001), one has to at least consider whether there is a causal relationship between these observations (Hipkiss, 2007a).

Carnosine (400 mg/day) together with omega-3-fatty acids (eicosa-pentaenonic acid) was employed in a study of dietary effects on dyslexic children. In this study there were no significant effects of the dietary supplements on a range of language skills and behavior problems (Kairaluoma et al., 2008).

There is some evidence that carnosine supplementation can restore sense of taste in humans. A report from Japan states that polaprezinc (a zinc–carnosine complex) is frequently effective in treating patients experiencing taste disorders (Ikeda et al., 2005). Experiments with zinc-deficient rats showed that polaprezinc was effective in restoration of taste bud proliferation (Hamano et al., 2006).

Polaprezinc has also been shown to be beneficial in treatment of ulcers and other gut lesions (discussed above) and in inhibiting some of the changes surrounding osteoporosis (also discussed above).

XXXX. WOULD VEGETARIANS BENEFIT FROM CARNOSINE SUPPLEMENTATION?

The possibility that vegetarian diets, deficient in carnosine, could be somewhat deleterious in the ability to suppress aldehyde-induced protein modification and AGE has been discussed (Hipkiss, 2005, 2006c). Indeed, Harris et al. (2007) showed that muscle carnosine levels were reduced by up 50% in vegetarian subjects. There is one report of

increased AGEs in vegetarian type-2 diabetics' sera (Krajcovicova-Kudlackova *et al.*, 2002). This may be due to the absence of carnosine in the vegetarian diet, but an alternative explanation could be that the increased fructose in the vegetarian diet increases AGE formation. It should be pointed out that many plants contain high levels of aldehyde scavengers which are probably present to protect plant proteins against glycating sugars such as fructose, and which could be exploited as dietary antiglycating agents.

XXXXI. DELETERIOUS EFFECTS OF CARNOSINE

Carnosine is usually regarded as being almost nontoxic (Sato *et al.*, 2008). However, there are some indications that the dipeptide can have deleterious effects. It has been known for a long time that humans with mutations in the gene coding for serum carnosinase show high levels of the dipeptide in their blood which is accompanied by neurological dysfunction (Gjessing *et al.*, 1990; Wassif *et al.*, 1994; Willi *et al.*, 1997). This may suggest that elevated serum carnosine or a failure to cleave the dipeptide elsewhere has deleterious effects, although there have been claims made that elevated levels of serum carnosine is not in itself a problem. There is one rather obvious possible way in which carnosine could be deleterious should the dipeptide prove to be an inhibitor of serum transglutaminase activity (Hipkiss, 2007a). Inhibition of this enzyme would suppress the development of cross-linking between fibrin molecules and could therefore compromise blood clotting. However, this remains a speculation at this stage.

There is one report suggesting that carnosine affects spermatogenesis in senescence-accelerated mice (SAMP1), reducing cell yield and increasing destructive changes in spermatogenic epithelium in the testicular tubules (Gopko *et al.*, 2005). However, the same research group had earlier stated (Zakhidov *et al.*, 2002) that carnosine did not modify the incidence of chromosome mutations in spermatogenic cells in these animals. There is also a paradoxical situation in the aging-resistant SAMR1 mice. In 2002 it was stated that carnosine increased the count of aberrant spermatogonia in the SAMR1 animals (Zakhidov *et al.*, 2002), whereas it was later reported that in these animals, carnosine treatment resulted in no increase in the incidence of aberrant spermatogia (Gopko *et al.*, 2005). Hence the significance of these observations is uncertain, but there have been no other reports of deleterious effects of carnosine. Nevertheless, it cannot be arbitrarily assumed that carnosine may not be without toxicity in some systems or organs.

XXXXII. CONCLUSIONS

Studies using model systems, cell culture, and animals have indicated that carnosine possesses a range of potential homeostatic functions which together may help to suppress many of the biochemical changes to macro-molecules which accompany aging and a number of related pathological conditions. Especially relevant is carnosine's carbonyl-scavenging ability which may prove to be particularly important in suppressing formation of protein carbonyls and those cross-linked protein species which inhibit proteasomal elimination of altered proteins. Carnosine may also stimulate nitric oxide synthesis and thereby increase proteasome activity, as well as upregulate synthesis of another protease, OPH. There is also some evidence that carnosine's other properties could also contribute to its antiaging activities include antioxidant, wound healing agent, aldehyde scavenger (including methylglyoxal), copper and zinc chelator, heat-shock protein inducer, anti-inflammatory agent, and antiepileptic agent. Much more work is required to explore all these proposals and speculations. As aging does appear to be multifactorially controlled, it is perhaps not unsurprising that a pluripotent agent such as carnosine might exert antiaging effects via more than one mode of action.

Evidence of carnosine's efficacy towards human health is relatively sparse in comparison with the range of effects observed in model systems and in animals. The most intensely investigated is cataractogenesis, although predominantly undertaken by a single research group. There is also relatively strong evidence that, in its zinc complex form (polapre-zinc), carnosine has positive effects on in repair of gut lesions such as ulcers. While a good case can be made for the use of carnosine in alleviating the deleterious effects of ischemic conditions, especially stroke-related events, there is little or no direct evidence as yet that the dipeptide is efficacious in human patients. This is also the position for the neurodegenerative diseases, AD and PD, although there is evidence from one research group showing that carnosine does have beneficial effects on Parkinson's patients undergoing treatment with L-dopa. The beneficial effects of carnosine on children with autistic spectrum disorders have been described by only one research group, so this needs verification. While a case can be made that carnosine may be useful in controlling the secondary complications associated with type-2 diabetes, there is no direct evidence that increased carnosine consumption suppresses their development in humans.

Clearly, much more work is required to verify or refute the many proposals made of carnosine's efficacy towards human health. Whether such studies will be undertaken is in doubt simply because of the non-patentability of the molecule and therefore it is unlikely to generate large

monetary benefit to any company or institution. However, if one were to try to determine whether carnosine does prevent many of the unpleasant effects of ''hangovers'' following excess ethanol consumption, one would anticipate that there would be no difficulty in finding volunteers. Science can sometimes be fun as well as intellectually challenging.

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