COMPOUND MONOGRAPH

L-ERGOTHIONEINE

Revision III

L-Ergothioneine

Abstract Summary

L-Ergothioneine (LE) is a unique, naturally occurring antioxidant that is abundant in most plants and animals. LE cannot be synthesized by humans and therefore is available only from dietary sources. The avidity by which dietary LE is assimilated by tissues, the specific and significant effects it has on cellular processes, and the degree to which it is conserved by cells suggest an important physiological role for this molecule. It has been shown that LE plays a dual role in both energy regulation and in protecting cells from oxidative damage.

High concentrations of LE are found in a number of organ systems including liver, kidney, the eye, seminal fluid, and erythrocytes. The biological significance of LE is only now beginning to be fully understood.

OXIS International, Inc. (OXIS) was the first company to develop a patented, synthetic process for the manufacture of pure l-(+)-ergothioneine. The company is now actively engaged in commercial applications for this natural antioxidant compound as a dietary supplement. This report is in support of that application.

A. Current State of Science

1. Origin and Biosynthesis

LE has intrigued biochemists since its discovery in 1909 (1,2), in a fungal contaminant of rye grain, it was later found to be highly concentrated in the red blood cells of most animals and aggressively conserved by the body. Over the years attempts have been made to characterize the its origin, fate and function (2-4). Early attempts to identify LE as a vitamin were discontinued due to the lack of a well-defined animal model of LE deficiency, but this role has been suggested by some authors (5).

One role clearly elucidated for LE is that of a natural intracellular antioxidant (4,6) similar in many aspects to the major cytoplasmic thiol, glutathione (GSH). Several additional biochemical and biological activities have been attributed to LE but its physiological role beyond being an antioxidant must be clarified (7,8). It does appear to have a significant role in maintaining the function of erythrocytes as well as protecting them from oxidative damage (9,10). The ability of LE to protect hemoproteins such as hemoglobin within erythrocytes (11,12) against oxidation probably could explain the millimolar concentrations seen in these cells. The avidity by which dietary l-ergothioneine is incorporated into tissues, the tenacity with which it is retained and its unique non-uniform pattern of tissue distribution serve to support the physiological importance of this molecule.

Over the years, LE research has been limited by the lack of availability of a commercial source of pure synthetic material. Recently, researchers at OXIS International have successfully developed the first patented, synthetic process for the industrial preparation of pure LE (13). The efficacy, clinical utility and commercial viability of other natural antioxidants in numerous disease states and in various OTC applications (cosmetics, preservatives, dietary supplements, etc.) are well known. Thus, this “recently rediscovered” natural antioxidant represents a new opportunity for development in areas where natural

antioxidants have been commercially successful. In addition, because of its unique biological properties, LE holds potential for use in a wide array of oxidative stress-induced disorders.

LE is synthesized by fungi and mycobacteria in soil where it is readily absorbed by plants through their roots (2). In these micro-organisms it is biosynthesized from hercynine and cysteine (2,14, 82-83). Cysteine is the source of the sulfhydryl, and the introduction of sulfur is the last step in the pathway (83).To date no mutant of these organisms has been identified which is lacking in LE biosynthesis.

No evidence exists for the direct biosynthesis of LE in animals or plants despite numerous studies attempting to identify a synthetic pathway (2,15,16,17,18). It seems likely that fungi are the source of most or all of the LE in plants and animals. It may be ingested either directly such as mushrooms or plants in animals, or indirectly by assimilation by plants from the soil (2). Hence humans are auxotrophic for this compound and therefore must assimilate it through dietary intake of plants and/or animal foodstuffs.

2. Biological distribution

LE is a ubiquitous compound found in most animals and plants. The literature values for LE in various sources has been hindered by a lack of consistency in the methods used. While the molecule has been studied for almost a century, only recently have specific and sensitive methods been employed. There still remains much work to be done to paint an accurate picture of the levels of LE across a wide spectrum of source materials. Table I summarizes the distribution of LE in various tissues for 4 animal species studied from Melville done in the 1950s (2).

Table I: Concentrations of LE in various animal tissues.

Tissue	Rat	Rabbit	Dog	Cat
Liver	13.3	0.3	0.9	2.7
RBC	10.4	10	6.6	2.9
Kidney	4.3	0.3	1.6	3.1
Heart	1.5	2.7	8.9	0.0
Lungs	1.5	0.3	0.6	0.8
Spleen	1.1	1.0	1.1	---
Testes	0.0	0.1	0.0	0.0
Muscle	0.7	---	---	---
Intestine	0.6	---	---	---
Stomach	0.4	---	---	---
Plasma	0.0	---	---	---

Values expressed as mgs/100gm fresh tissue.

Millimolar or submillimolar concentrations of LE have also been found in the bone marrow (19), cataract-free lens (20), and seminal fluid (21,22). While brain tissue is not reported in Melville's chart, Briggs (81) did an extensive study of LE distribution in Ox brain and determined there were values that ranged from 0.36 to 0.03 mgs/100gm depending upon the brain region examined. As can been seen, even on a relative basis, there is a tremendous range of distribution of LE across many species and the organ systems within the species. The existence of this data would support an evolved selective biological distribution of the molecule. The distribution pattern can be further evidence for a functional attribute. LE is not known to be a significant energy source, or have a structural role, therefore the implication is that

LE plays an important role in homeostasis as a regulator or effector. Its role as antioxidant is that of a maintenance function.

LE is preferentially distributed to organ systems that are exposed to a high degree of oxidative stress. Blood concentrations of LE in almost every species investigated are in near millimolar range as shown in Table II (2).

Table II: Concentration of l-ergothioneine in the blood of various animals.
(mg/100ml blood)

Species	Ergothioneine
Man	1 – 4
Rat	1 – 6
Rabbit	1 – 10
G. Pig	1 - 4
Cat	0.5 – 2
Dog	3 – 6
Ox	0.5 – 2
Pig	3 – 27
Sheep	2 – 6
Fowl	2 – 10

3. Biochemistry

Chemically, LE corresponds to the betaine of 2-thio-L-histidine. Although various synthetic compounds of this chemical class exist, LE is the only naturally occurring 2-thio-imidazole amino acid known to date. In aqueous solution, the tautomeric 2-thio-imidazole exists predominantly in the thione form. This explains why, unlike other alkylmercaptan antioxidants such as GSH, at physiologic pH LE does not auto-oxidize and is therefore very stable in aqueous solution.

The compound is extremely hydrophilic with a solubility limit of 0.9 M at room temperature (23). It is an excellent chelator of divalent metals especially copper and zinc (24,25) and is remarkably stable to strong alkali, properties which further differentiate it from other biological thiols.

Over the years several isolation techniques have been developed which employed starting materials such as blood, ergot or grains (2,26,27). Pig blood has the highest know amount of l-ergothioneine, but yields only 60 to 100mg of l-ergothioneine per liter of blood processed (2,26). Various fungal and mycobacterial sources are available for the production LE but the yields are too low to be commercially viable for an industrial production (28).

The synthesis of the natural l-isomer of LE has proven to be quite difficult. Several attempts to synthesize LE (29,30,31) have failed to achieve satisfactory yields, were not industrially viable or resulted in partial (32) or complete racemization (33). OXIS International has developed the first efficient, commercially viable synthesis of LE resulting in high yield (34) based on a new method of sulfur introduction into an imidazole ring (35).

Various methods have been employed to perform chemical analysis of LE. There are no commercially available assays for LE. Early seed methods (58) were improved upon by a spectrophotometric assay developed by Carlsson (73) which eliminated cross reacting thiols and ascorbic

acid with a Cu++ catalysed oxidation and the reaction of the non-labile LE with the reagent 2,2' dipyridyl disulfide. HPLC methods have been the preferred means to accurately determine LE. Several methods have been published (59, 74, 75). Oxis has developed an internal unpublished assay that will be highly specific, sensitive, and will amenable to high throughput analysis of samples from plant and animal origin. In addition Oxis will validate identification of purified samples with a direct comparison of pure synthetic LE by Mass spectral and Optical Rotatory Dispersion analysis.

The role of LE as an antioxidant and cellular protectant have been well documented (2,4). However, the unusual physico-chemical properties of LE and its preferential localizations within certain cells such as erythrocytes make it unique among naturally occurring antioxidants. The antioxidant properties of LE appear to be related to at least four activities which include the molecules ability to:

- Scavenge directly reactive oxygen species;
- Chelate various divalent metallic cations;
- Activate antioxidant enzymes such as glutathione peroxidase (Se-GPx) and MnSOD and to inhibit superoxide-generating enzymes such as NADPH-Cytochrome c reductase;
- Affect the oxidation of various hemoproteins such as hemoglobin and myoglobin.

In physiological concentrations, LE exhibits potent diffusion-controlled inactivation of hydroxyl radical (32, 84) and prevention of singlet oxygen production (38,39). It does not function as a direct scavenger of superoxide anion, hydrogen peroxide or lipid peroxides. It also differs significantly from natural thiol-containing antioxidants in that it does not stimulate lipid peroxidation in the presence of ferric ions.

LE is a powerful scavenger of hypochlorous acid (HOCI). Although many compounds can react with hypochlorous acid, few do so rapidly enough to be biologically meaningful. Alpha1-protease inhibitor (API), the major inhibitor of serine proteases such as elastase, is an especially sensitive target of hypochlorous acid. Studies have shown that physiological concentrations of LE protect very efficiently API against HOCI-induced inactivation (6). However, since neutrophils are the principal source of hypochlorous acid in the body, one role for LE may be to protect circulating erythrocytes from the damage induced by neutrophils during normal function or pathologic inflammation.

Peroxynitrite, formed endogenously by the diffusion-limited reaction of nitric oxide with superoxide, is a potent oxidant which has been implicated in the pathophysiology of inflammation, ischemia-reperfusion injury, atherosclerosis, acute lung injury and sepsis. It has been shown that LE inhibits the peroxynitrite-mediated oxidation of amino acids such as the nitration of tyrosine and protects against the peroxynitrite-induced inactivation of alpha-1-antiproteinase (72).

Divalent metals such as iron, copper and zinc have been shown to participate in the production of destructive reactive oxygen species (ROS). Iron-induced conversion of hydrogen peroxide to the more damaging hydroxyl radical via the Fenton reaction is thought to be one of the primary mechanisms for initiation of free radical mediated tissue damage. In fact, iron is frequently used in many in vitro models as a means of generating free radicals to induce lipid peroxidation. In addition, copper is routinely employed as a catalyst for the evaluation of anti-lipidemic agents and their ability to inhibit LDL oxidation, which is believed to be the initiating event responsible for atherosclerotic plaque development. Compounds capable of complexing these metals (such as desferoxamine) have been shown to be highly effective in ameliorating various oxidative stress related diseases. However, their usefulness has been generally limited because of toxicological problems. In contrast, LE, a natural antioxidant found in human tissues, as a metal chelator (24,25), should have minimal toxicity.

LE inhibits the NADPH-dependent enzymatic lipid peroxidation of hepatic microsomes after either NADPH or ascorbic acid challenge (39). Japanese researchers have found that LE markedly increased glutathione peroxidase and glutathione reductase activities in mouse liver cytosol fractions (39). Additionally mitochrondrial Mn-superoxide dismutase activity was nearly doubled by LE at a concentration of 12.5 mM but no increased activity of the cytosolic form of Cu/Zn SOD was noted. Thus, LE may also exert a significant biological benefit by stimulating cellular antioxidant systems against oxidative challenge. It remains to be determined if the enzymatic effects of LE are a consequence of direct enzyme stimulation and/or a consequence of the conservation if glutathione levels.

Another source of ROS in vivo is the exposure of hemoproteins such as hemoglobin or myoglobin to hydrogen peroxide (41,42,43). For example oxyhemoglobin reacts with hydrogen peroxide to generate a high oxidation state iron species (Fe^{IV}=O) called ferryl-hemoglobin. This species plays a critical role in the lipid peroxidation of erythrocytes. Similarly myoglobin exposure to hydrogen peroxide causes oxidation of lipid peroxidation of erythrocytes. Similarly myoglobin exposure to hydrogen peroxide causes oxidation of lipid membranes and contributes to the ischemia/reperfusion injury noted in ischemic heart or muscle (44). It has been shown that LE reduces the ferryl-myoglobin into metmyoglobin and by this way inhibits the lipid peroxidation (44) and protects against ischemia-reperfusion injury (45). Studies using the Langendorff model in rats have demonstrated an interaction of ferryl-myoglobin with LE in tissues, showing that LE (0.1mM) protected the isolated rat heart against reperfusion injury as measured by LDH release. In a similar study on isolated rabbit heart, LE failed to protect against ischemia-reperfusion injury (46). This discrepancy may be explained by several differences in the experimental models including species, perfusion methodology and duration of ischemia. However, OXIS researchers using LDH and CPK as measure of cardiac damage have confirmed the earlier isolated rat heart findings for LE.

Aruoma (71) tested LE for its ability to inhibit cell death caused by H2O2 and to inhibit DNA oxidation by peroxynitrite in a human neuronal hybridoma cell line in culture.

LE demonstrates secondary activities, which are independent of its antioxidant properties. Data suggest that it plays a role in the regulation of the energy requirements of erythrocytes (47). Since erythrocytes do not contain any mitochondria, they utilize the pentose-phosphate shunt to meet their energy requirements, resulting in the formation of lactic acid. When LE is added to hemolyzed erythrocytes or erythrocyte suspensions, increased lactate production is noted along with a concomitant decrease in intermediate glucose byproducts such as glucose-6-phosphate and fructose-6-phosphate. These biochemical changes are consistent with energy production by LE within erythrocytes. When LE was administered in the diet to rats it prevented a 40% starvation-induced diminution or erythrocytes lactate level that was noted in the control animals. In vitro incubation studies of human platelets demonstrated LE involvement in cellular energy production with increases in CO_2 production from pyruvate noted concomitantly with a decreased production of lactate. These effects were similar to those noted for carnitine suggesting that LE can also stimulate energy production in cells using normal cellular respiratory pathways. (48). Therefore LE may be required for these cells to maintain normal metabolic function, especially when exposed to high oxidative challenges. This may also explain why LE is selectively concentrated in the mitochondria. By utilizing the carnitine membrane shuttle, LE can enter from the outer to the inner membrane, possibly by a competitive binding mechanism.

4. Pharmacology

It has been shown that LE protected rats in vivo against hepatic injury associated with ethionine administration (a form of damage which occurs via formation of lipid peroxides). The hepatic injury

produced after a single injection of ethionine was inhibited and hepatic lipid peroxide formation was reduced by pre-administration of LE (8mg/100gm body weight) for 7 days. A 40% increase in hepatic lipid peroxidation was noted in partially LE-deficient rats (<1nmol/gm liver) as compared with dietary-augmented animals (1400nmol/gm liver) (49).

The lens of the eye undergoes extensive oxidative challenge on a continuous basis. Cataract formation has been shown to result from a cumulative exposure of the lens to UV radiation. The resulting production of ROS over time depletes the antioxidant defenses normally present in the eye resulting in the gradual formation of cataracts. LE has been found in high concentrations in the normal human eye (20) where it presumably functions as a protecting antioxidant. Table III summarizes the finding from a study (20) which examined the relationship between LE concentration and cataract development.

Table III: The concentration of LE in the lens of the human eye as a function of cataract development.

Stage of Cataract	LE	Number
Normal	115.7 +/- 6.3	N=10
Immature:		
Nuclear	94.2 +/- 7.3	N=20
Cortical	79.4 +/- 11.7	N=20
Mature	71.7 +/- 13.7	N=50
Hypermature	60.8 +/- 9.8	N=25

A clear reduction in LE concentration is evident over the course of cataract development. The greater decrease in LE noted in the cortical type of immature cataract is apparently related to the higher metabolic state associated with the cortex relative to the nucleus. The researcher also noted a progressive loss in LE concentrations as opacities increased.

LE has also been studied in various radiation-induced damage models (50,51, 84). UV –induced skin damage is known to be mediated via formation of various ROS, including singlet molecular oxygen, hydroxyl radical and superoxide. The effect of LE on inactivation of singlet oxygen was studied relative to other biological thiols (52). As compared to cysteine, N-acetyl-cysteine, glutathione and other synthetic mercaptans, LE was found to have the greatest affinity for singlet oxygen, i.e. 10 fold greater than glutathione. Although LE failed to protect mouse skin in vivo (53), it seems likely that it was not delivered through the stratum corneum. Ergothioneine was found to be a very potent radioprotector even at low concentrations in an in vitro study (85). Several patents have included LE as an active UV protectant.

Studies have shown that LE has a critical protective role in seminal fluid (21,22). LE is the predominant sulfhydryl in human, horse and pig semen. Its role is evidently to protect spermatozoa from oxidative stress, given the exceptionally high metabolic rate in sperm. LE, as consequence of its antioxidant properties, counteracts the effects of hydrogen peroxide on spermatozoa viability and survival while also enhancing the viability of sperm during storage.

Animal studies have shown that as little as 1 part per 100,000 of LE in the diet can produce measurable changes in circulating whole blood levels (54). Following intravenous administration, pharmacokinetic studies (2) revealed a rapid disappearance of LE from the plasma into the organs, principally the liver. Neither hepatectomy nor nephrectomy altered this disappearance suggesting that tissue uptake rather than excretion occurred. These studies suggest that LE is rapidly absorbed and

assimilated by various tissues. Complete depletion of LE from tissue stores has proven to be difficult (55,56) even after extensive periods of starvation. The whole body half-life of LE in the rat is approximately 1 month (57). In rats, one study measured the fecal and urine loss of ergothionine, and calculated the absorptive gain per day on a controlled diet of ergthioneine supplementation. The gain was 20ug/day for a 225g rat, while the loss was 2 ug/per day ;indicating a 90% accumulation rate. For humans this would equal a retention rate of about 6 mg per day for 150 lb adult (54).

LE uptake into red blood cells is believed to occur during erythropoiesis and it appears to remain present within the cell for its entire lifetime (2,4,10,58). These data suggest that various processes exist within the erythrocyte for its conservation and/or regeneration. Over the normal 120-day life span of the erythrocyte, LE concentrations gradually decrease (9,59) probably as a consequence of cumulative oxidative exposure. This gradual decline in LE concentrations within the erythrocyte, despite normal dietary intake, plus the high LE concentrations found in bone marrow, add credence to the theory that LE enters erythrocytes during hematopoiesis. Numerous studies have found that only minute amounts (<1%) of LE can directly penetrate the erythrocyte membrane (2,10,58,59,60). Although the mechanism of uptake has not been elucidated, the fact that erythrocytes from most species contain high concentrations of LE suggests that one of its principal biological activities is associated with erythrocyte function and regulation. LE penetration into seminal fluid and most other tissues besides erythrocytes is rapid.

No documented negative side effects or know toxic pathways have been reported as a result of LE administration. Kunisaki speculated that intake of nitrites in the diet may enhance the formation of nitrosamines (86). No physiological nitrosamine product was ever isolated or levels measured to support the theory.

5. Metabolism

The metabolic fate of LE in humans has never been studied. Animal studies have been performed mainly in rat and rabbit models. As was stated earlier LE given in the diet at low concentrations is very efficiently absorbed and retained in the rat. However many studies have been performed using bolus injections intraperitonealy or directly into the blood. These conditions most certainly will not yield the same metabolic fate as the low dose dietary route of entry.

Unfortunately there is a lack of uniformity in procedures used to study the fate of radiolabled LE. However the results of several researchers provide some insight into the transformations that are possible. The metabolism of ^{14}C-dLE was examined in the rat (61). Apparently the most important step in LE metabolism is the loss of the thiol group. The liver was identified as the principal site of LE metabolism where it is converted to the non-thiol containing derivative hercynine, which is subsequently excreted. This conclusion is supported by studies with ^{35}S-ergothioneine (15) where 35-65% of the sulfur label was recovered in the urine as free sulfate. Urinary excretion is the principal route of bolus injected LE elimination, with 60% of the dose being recovered in approximately 6 hours. About one-third of the urine products were identified as LE and another one-third as hercynine.

Microbial metabolism may occur to some extent in the gut. However efforts to measure synthesis, even at very high levels of radioactivity, were unable to measure any significant incorporation of radiolabled histidine into isolated LE. Because humans do have the genetic machinery to synthesize LE, the species has not evolved a specialized degradation pathway. Indeed, instead what has evolved are systems to sequester and maintain exogenously derived LE. It is not clear exactly what transport systems exist. The mitochondrial carnitine shuttle may be one of those systems.

The metabolism and biochemistry of LE within the context of its function as an antioxidant is still being explored. Many groups have looked at LE and its interactions with free radicals . Asmus (66) found

that LE interacts effectively with oxidizing radicals (hydroxyl, azide, and carbontetrachlorideperoxide), and in the presence of ascorbate is regenerated back. Ascorbate performs a similar function in converting tocopherol quinone back to Vitamin E. In an animal experimental model of diabetes, Aruoma and coworkers have shown that antioxidants (BHT, Vitamin E, Vitamin C) decrease the rate of embryo malformations (67). The same group was also able to show that LE can also act to reverse the developmental defects to almost that of the control group (68).

6.Toxicology

Extensive animal toxicity studies with LE have not been reported. However, results from numerous experiments have produced no deleterious effects following LE ingestion in either humans or animals. LE is present in various foodstuffs (2) and is readily absorbed after ingestion as part of a normal diet. The risk of any serious toxicity associated with the compound is probably minimal especially given its high circulating and tissue levels. Abbreviated pharmacological studies (62,63) originally conducted in the 1920's and 1930's failed to show any deleterious effects of LE at physiologically relevant concentrations. The carcinogenic potential of the compound is apparently low with several investigators reporting that LE actually protects against mutagen production (64,65).

Health Benefits and Disease Prevention

Aruoma's group has shown that LE inhibits both H2O2 and Tumor necrosis factor-alpha-mediated imposed oxidative stress in an IL-8-chloramphenicaol acetyl transferase (CAT) reporter system in A549 cells. This anti-inflamatory response was found to be due to a lowering of transcription NF-kB and Activator Protein-1, resulting in abolishing the transcription activation of the pro-inflamatory cytokine Interleukin-8 (69).

Aruoma has also shown a neuroprotective effect of LE in the rat retina in an in vivo N-methyl-D – aspartate excitotoxicity system (70). Cell counts revealed that 81% of ganglion cells and 43% of non-ganglion cells were lost as a result of the treatment. In rats treated with LE, the losses were dropped to 44% and 31% respectively. Interesting they also measured Ameloid Precursor Protein (APP), and found significant decreases. The protein has been associated with Alzheimer's Disease. A similar protective effect was found against the oxidative base modifications induced by peroxynitrite on calf-thymus DNA , and nitration of tyrosine and inactivation of alpha-1-antiproteinase (72).

A study of 115 subjects with various cataractous lense was compared with 10 normal lenses (76). The quantity of ergothioneine (expressed as mg LE/100 gm) was markedly reduced in all four types of cataractous lenses (immature nuclear 94.2 , Cortical 79.6, Matue 71.7, hypermature 60.8) compared to controls (115.7). The reduction may reflect an inability to deal with oxidative stress since GSH levels are lowered as well (76). Aruoma (77) has data to show that when rats are injected intraperitoneally with LE, it can be incorporated into the retina and reverse the effects of NMDA toxicity. These findings in total indicate that LE may play some protective role in the formation, and potential reversal of cataracts.

Diabetes Melitus has been associated with oxidative stress (88-89). Use of antioxidants may have a profound positive influence in controlling the oxidative stress problems associated with diabetes. An early study showed that some diabetic patients had elevated levels of LE (78). This lead Epand to speculate that LE could be inducing diabetes through chelation of zinc (79). Later experiments performed by Epand found there to be no statistical significance between blood levels of LE between diabetic and non-diabetics (80).

Scientific support of potential claims

1. Old dietary supplement, dosage levels.

Oxis believes that the use of LE as an old dietary supplement is because it has been documented to have been in the food supply in significant quantities for at least a century prior to 1994, and the DSHEA (1, 2). The synthetic molecule is identical to that of the naturally occurring molecule, as proven by Oxis through chemical analysis (13, 34).

Isolation of LE from oats (17 mg/kg), mushrooms (100-1000mg/kg), and corn has been shown by Melville (2). Meat also contains ergothionine, but there have not been published composition data derived from commercial sources. It is an a safe assumption that the value will approximate that of blood and muscle, which would range from 5-20 mg/Kg. LE has been shown to be very stable to heat and pH ranges (59), and would survive the normal conditions of cooking

It is difficult to determine how much LE is being taken in by the average consumer today. This will undoubtedly vary widely from culture and region. If we use only the United States, and make some conservative assumptions about the types and quantities of foods that are consumed, we can develop some range estimates based on food composition and retention/absorption data in the rat. If the average adult consumes 1 pound (approximately 0.5 Kg) of LE containing foodstuff per day, and the average amount is the average of cereal (oat value of 17 mg/kg) and meat (10 mg/Kg), we arrive at an amount of [(17 +10)/2] mg/kg x 0.5 Kg, or approximately 7 mg per day. For a 150 lb individual this would calculate to 104 ug LE/kg-day body weight. The rat absorption study of Mayumi et al. (59) concluded that a 225 gm rat absorbed 90% of the LE provided in the diet, which was 20 ug/day, or 89 ug LE/kg-day. Thus, the values are very similar. From this we can conservatively say that if the human absorption capacity is similar to that found in the rat, then the vast majority of ingested LE is retained. Therefore supplementation on the order of 5-10 mg per day would certainly be within an order of magnitude, and more likely a factor of 2-3 of what the average human adult is consuming in their diet already.

Mayumi concluded that it would take the rat approximately 2 months to double the total body amount of LE at 89 ug LE/kg-day supplementation rate. If the same is true in humans, then we can say that this slow rate of accumulation would allow a very comfortable adjustment period for determining any potential side effects due to the supplementation. A two-month adaptation period is quite long compared to most pharmaceuticals. For example, antidepressants will usually ramp from half to full dosage up over a period of 30 days.

Oxis is presently undertaking a wider scope of analysis of foodstuffs for LE. These data will allow more precise estimations of LE dietary intake. Melville points out however that because LE is not synthesized by plants, but is actively transported from the soil through the roots, there may very well exist a wide variation in the amount of LE in selected vegetable sources, depending upon the soil conditions of the region. This variation will also affect the levels of LE found in meat sources as well.

2. Specialized Unique Antioxidant

As previously described, LE is a highly protective, nontoxic, naturally occurring antioxidant that is not easily auto-oxidizable in aqueous solutions. It is readily water soluble, reaches near millimolar concentrations in selected tissues, and stimulates the natural antioxidant defenses within cells. The benefits of natural antioxidants such as vitamin C and vitamin E in cancer, aging and general health are well known. Newer natural antioxidants such as pyrogallol, lipoic acid and ubiquinone are now being

introduced into the market. LE is unique among antioxidants in that it chelates heavy metal, while protecting cells (principally erythrocytes) from ROS damage. .

3. Dermal Protectant/Anti-aging: The role of free radicals in ultraviolet light induced skin damage, and the role of UVB radiation in skin cancer is well known. LE, because of its ability to minimize the formation of various ROS and to protect cells from radiation induced damage, is currently being evaluated in novel liposomal delivery systems. Since LE is a natural product, the goal of this program is to develop OTC sunscreens and/or protective cosmetics.

4. Ophthalmic benefit: The observation that the eye contains extremely high concentrations of LE that decrease during cataract development suggest that LE plays a critical role in the protection of the eye. The effect of UV radiation on the eye and its association with cataracts is well known. Oxis, in collaboration with researchers in Paris, is evaluating the role of LE in the eye. The aim is to develop an ophthalmic product to replenish the loss of LE noted during cataract development. The aqueous solubility of LE and its stability offer major advantages since the ideal ophthalmic should be available to topical administration.

5. Energy enhancer: Evidence from several sources points to a positive effect of ergothionine in increasing the availability of cellular energy sources. Kawano (47) speculated that LE might stimulate Phospohfructokinase activity or increase glucose absorption in erythrocytes as a way to explain the significant increase in glycolytic activity measured after addition of 1 mM LE to intact human red cell cells in vitro. A similar report was made by Chiba (87), but the compound was described only as a synthetic sulfhydryl compound. As was discussed in the Biochemisrty section there is good evidence for increased respiration in platelets, with LE playing a role similar to carnitine in shuttling acyl-CoA derivatives across the mitochonrial membrane (48).

6. Organ Preservation: The availability of viable organs for transplantation is currently limiting the number of organ transplants that can be conducted in the US. Preservation of the available tissues and prolongation of their viability is an important determinant in both the ultimate success of the procedure and the number of patients that can receive transplants. Specifically, liver viability is limited to 8 hours, which severely limits the transport of these organs. Although, current preservation solutions are formulated to include antioxidants such as glutathione, the instability of these compounds (significant degradation begins to occur immediately after manufacture) limits their usefulness in protecting organs from oxidative damage. Glutathione even in refrigerated preservation solutions is readily oxidized, to its disulfide form. The later form is cytotoxic and also facilitates inflammation-induced proteolysis. LE, a stable water-soluble thiol-containing antioxidant that also chelates metal ions, could be an ideal to replace glutathione in this mixture. .

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