Free Radical Biology & Medicine, Vol. 24, No. 6, pp. 1023–1039, 1998

0891-5849/98 $19.00 + .00

PII S0891-5849(97)00371-7

ELSEVIER

Review Article

α-LIPOIC ACID IN LIVER METABOLISM AND DISEASE

JUANITA BUSTAMANTE,* JOHN K. LODGE,* LUCIA MARCOCCI,* HANS J. TRITSCHLER,†
LESTER PACKER,* and BERTRAND H. RIHN*

*Membranes Bioenergetics Group, Department of Molecular and Cell Biology, University of California, Berkeley, CA, USA and †Asta Medica AG, Frankfurt-am-Main, Germany

(*Received* 8 *August* 1997; *Revised* 1 *October* 1997; *Accepted* 1 *October* 1997)

Abstract—R-α-Lipoic acid is found naturally occurring as a prosthetic group in α-keto acid dehydrogenase complexes of the mitochondria, and as such plays a fundamental role in metabolism. Although this has been known for decades, only recently has free supplemented α-lipoic acid been found to affect cellular metabolic processes in vitro, as it has the ability to alter the redox status of cells and interact with thiols and other antioxidants. Therefore, it appears that this compound has important therapeutic potential in conditions where oxidative stress is involved. Early case studies with α-lipoic acid were performed with little knowledge of the action of α-lipoic acid at a cellular level, but with the rationale that because the naturally occurring protein bound form of α-lipoic acid has a pivotal role in metabolism, that supplementation may have some beneficial effect. Such studies sought to evaluate the effect of supplemented α-lipoic acid, using low doses, on lipid or carbohydrate metabolism, but little or no effect was observed. A common response in these trials was an increase in glucose uptake, but increased plasma levels of pyruvate and lactate were also observed, suggesting that an inhibitory effect on the pyruvate dehydrogenase complex was occurring. During the same period, α-lipoic acid was also used as a therapeutic agent in a number of conditions relating to liver disease, including alcohol-induced damage, mushroom poisoning, metal intoxification, and CCl_4 poisoning. α-Lipoic acid supplementation was successful in the treatment for these conditions in many cases. Experimental studies and clinical trials in the last 5 years using high doses of α-lipoic acid (600 mg in humans) have provided new and consistent evidence for the therapeutic role of antioxidant α-lipoic acid in the treatment of insulin resistance and diabetic polyneuropathy. This new insight should encourage clinicians to use α-lipoic acid in diseases affecting liver in which oxidative stress is involved. © 1998 Elsevier Science Inc.

Keywords—α-Lipoic acid, Thioctic acid, Oxidative stress, Free radicals, Liver, Disease, Primary biliary cirrhosis

Address correspondence to: Lester Packer, Membranes Bioenergetics Group, Department of Molecular and Cell Biology, 251 Life Sciences Addition, University of California, Berkeley 94720-3200.

L.M. is on leave from the Department of Biochemical Sciences ''A. Rossi Fanelli'' and CNR Center of Molecular Biology, University of Rome ''La Sapienza'' Piazzale Aldo Moro 5, 00185 Rome, Italy.

B.H.R. is on leave from Institut National de Recherche et de Sécurité, Avenue de Bourgogne, 54500 Vandoeuvre, France.

Dr. Lester Packer is a professor in the Department of Molecular and Cell Biology at the University of California at Berkeley. He received a Ph.D. in Microbiology and Biochemistry from Yale University in 1956. At Berkeley he has been Professor of Physiology since 1961; Director of the Membrane Bioenergetics Group at the University of California at Berkeley, and a Senior Scientist at the Lawrence Berkeley Laboratory since 1972. His research in the area of biological oxidation and bioenergetics has emphasized studies on the role of oxidants and antioxidants in biological systems. Recent work concerns the prevention of oxidatively-induced injury in biological systems by antioxidants and how networking antioxidants such as vitamin E, C, and lipoic acid affect gene expression and cell regulation.

Dr. Bertrand Rihn is Head of the Laboratory of Experimental Carcinogenesis at the Institut National de Recherche et de Sécurité, Vandoeuvre, and Head of the PCR Laboratory, Centre Hospitalo-Universitaire Régional de Brabois, Vandoeuvre, France. He obtained an M.D. at the Université Louis Pasteur (Strasbourg) in 1982, and in 1991 received his Ph.D. His current research interests are in the development of nonradioactive methods for molecular biology, the development of new tools for in vivo experimental carcinogenesis, and the evaluation of carcinogenicity of chemical compounds.

Dr. Juanita Bustamante is a researcher at the Catedra de Fisicoquimica Facultad de Farmacia y Bioquimica, University of Buenos Aires, Argentina. She obtained her Ph.D. in Chemical Sciences at the University of Buenos Aires in 1991. Her research interests are in the role of antioxidants and oxidants in apoptosis.

Dr. John K. Lodge is a researcher in the Department of Molecular and Cell Biology at the University of California at Berkeley. He obtained his Ph.D. in Biological Chemistry at the University of London in 1993. His current research interests are in oxidative stress.

Dr. Lucia Marcocci is a researcher in the Dipartimento di Scienze Biochimiche ''A. Rossi Fanelli,'' at the Universita' di Roma ''La Sapienza.'' She received a Ph.D. in Biochemistry at the Universita' di Roma ''La Sapienza'' in 1991. Her research interests are in oxidative stress.

INTRODUCTION

α-Lipoic acid, a disulphide derivative of octanoic acid, has been known for decades to be a crucial prosthetic group of various cellular enzymatic complexes. Recently, α-lipoic acid has been characterized as an efficient antioxidant. It has been proposed to be a potential therapeutic agent in the treatment or prevention of different pathologies that may be related to an imbalance of the oxidoreductive cellular status. This occurs in the case of neurodegeneration, ischemia–reperfusion, polyneuropathy, diabetes, AIDS, and hepatic disorder status (see Packer et al.[1]).

Although various studies on experimental models, as well as on clinical trials, suggest a beneficial effect of α-lipoic acid in the treatment of neurodegenerative conditions, AIDS, and diabetes, the efficacy of α-lipoic acid in hepatic and hepatic-associated diseases is controversial.

To critically evaluate the potential effectiveness of α-lipoic acid in hepatic and hepatic-associated diseases, we will discuss information obtained from in vitro or in vivo experimental models on the role of endogenous or administered α-lipoic acid on physiological functions of cells, and review the available reports on its efficacy as a therapeutic agent when used in clinical trials.

UPTAKE, METABOLISM, AND DEGRADATION OF ALPHA-LIPOIC ACID

Various studies on the distribution of radioactivity in rat tissues after intraperitoneal or oral administration of *dl*-[^{14}C]- or [^{35}S]-lipoic acid led to the observation that α-lipoic acid is rapidly absorbed in the gut, taken up into various tissues where it is metabolically altered, and then excreted. After supplementation with α-lipoic acid for 5 weeks, free α-lipoic acid was found in various tissues, the highest being the heart.[2] When given to cells in vitro, α-lipoic acid is rapidly taken up by the cells and reduced to dihydrolipoic acid, which is released by the cell. A detailed study of the uptake of α-lipoic acid in hepatocytes was carried out by Peinado et al.[3] The biliary excretion of [^{35}S]-compounds after administration of [^{35}S]-α-lipoic acid showed that lipoate is transported into the hepatocyte, and not merely bound to the hepatocyte membrane. Thin-layer chromatography analysis showed that these compounds consist mainly of metabolic products not identical to lipoate. The hepatic uptake of α-lipoic acid, and the excretion of [^{35}S] compounds into the bile, were found to be simultaneously suppressed by the presence of medium chain fatty acids (octanoate), which suggests that lipoate and medium chain fatty acids are transported via the same translocater.[3] The lipoate uptake followed kinetics with saturable and nonsaturable components. The saturable component showed a K_m of 38 mM and predominates at concentrations below 75 mM. The authors suggested that hepatic lipoate uptake is carrier mediated at low concentrations, while at higher concentrations diffusion can become a major uptake pathway. The physiological concentration in the blood (80 ± 17 nM), is well below the K_m value;[4] therefore, normal cellular uptake would be via the transport mechanism.

Urinary excretion is maximal 3–6 h after administration of α-lipoic acid, suggesting that the clearance of lipoate metabolites from the rat is even faster than indicated in many studies.[5–7] It has been reported that approximately 45% of the radioactivity administered with isotopically labeled α-lipoic acid is excreted in the urine during the first 24 h, and only 3% is excreted in the feces.[6] In any case, several studies on its metabolism in humans and rats have shown that very little administered α-lipoic acid is excreted in the unaltered form.[5–8] After 4 h of intraperitoneal or oral administration of *dl*-[^{14}C]-lipoic acid, the highest amount of radioactivity was observed in the liver, but 24 h later, radioactivity was found in the skeletal muscle.[6] Other studies showed that after 5 to 200 mM [^{35}S]-lipoate infusion into the liver, about 7 mmol/g liver of lipoate or lipoate metabolites were found.[3] This demonstrates that the liver has a high capacity for uptake and accumulation of these compounds, in accordance with the in vivo studies. It has been demonstrated that catabolic pathways involved in lipoate metabolism are largely through β-oxidation of the valeric acid side chain, but the carbon skeleton of the dithioline ring portion is much more resistant to alteration.[7] Such metabolites include the short chain analogues bisnorlipoate and tetranorlipoate, which were found at 0.5 and 0.7% of the injected radioactivity. A hydroxy derivative of β-hydroxybisnorlipoate was also found and represented 4.9% of the injected radioactivity. Such low levels of metabolites suggests that the fate of the majority of supplemented α-lipoic acid is in the unaltered form. Other metabolites have also been suggested, because unknown compounds were found to react with dinitrophenylhydrazine, implying the presence of carbonyl groups.

ROLE OF ALPHA-LIPOIC ACID ON PHYSIOLOGICAL FUNCTIONS OF EUKARYOTIC CELLS

The unique physico-chemical properties of α-lipoic acid make it a powerful and reactive biological molecule, chosen by evolution to carry out biochemical reactions crucial for oxidative metabolism, and shown by researchers to modulate various cellular functions (Fig. 1).

A strain on the oxidized dithiolane ring cyclic structure of α-lipoic acid (3 to 6 kcal/mol) gives the molecule

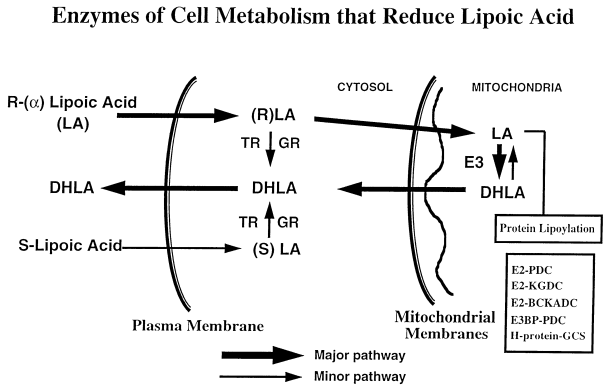

Fig. 1. The intracellular fate of protein-bound andexogenously supplemented α-lipoic. Naturally occurring α-lipoic acid, present in the five mitochondrial proteins as described, is interconvertable with the reduced form DHLA by the enzyme E_3, which requires NADH/NAD^+. Supplemented α-lipoic acid enters the cell and is reduced by the cytosolic enzymes glutathione reductase and thioredoxin reductase at the expense of NADH, and also the mitochondrial enzyme E_3. The activities of each enzyme is dependent upon the form of lipoic acid and the tissue (courtesy of Dr. Mulchand S. Patel and Dr. Lester Packer).

a high tendency for reduction according to environmental conditions. On the other hand, the low negative redox potential ($E_0' = -0.29$ V) makes the lipoic acid/dihydrolipoic acid couple a strong reductant. Similar to other vicinal thiols, dihydrolipoic acid (which has a high pK value for the —SH, around 10.7) is more easily oxidized in comparison with monothiols, leading to high activity in —SH/S—S— interchange reactions.[9] Furthermore, both α-lipoic acid and dihydrolipoic acid have high hydrophobicity, which allow them to permeate biological membranes at a high rate.

Described below are the biological properties of α-lipoic acid as a prosthetic group bound to proteins and as a free molecule administered to cellular systems under in vitro or in vivo conditions.

Protein-bound α-lipoic acid

α-Lipoic acid is present as a cofactor in α-keto-acid dehydrogenases and in the glycine cleavage system. These enzyme complexes are involved in the metabolic pathways of pyruvate oxidation, the citric acid cycle, and amino acid degradation and biosynthesis.

α-Keto acid dehydrogenase complexes. The α-keto acid dehydrogenase complexes, i.e., the pyruvate dehydrogenase complex (PDC), the α-ketoglutarate dehydrogenase complex (α-KGDC), and the branched chain α-ketoacid dehydrogenase complex (BCKADC) constitute an almost ubiquitous family of enzymes (see Patel et al.[10] for a review). They are located in the mitochondrial matrix, associated with the inner membrane, and catalyze the oxidative decarboxylation of various α-keto-acid substrates (pyruvate, α-keto-glutarate and the short branched-chain α-keto-acids produced by transamination of leucine, isoleucine, and valine) to the corresponding acyl-CoA forms (acetyl-CoA, succinyl-CoA, and isovaleryl-CoA, respectively, Table 1) forming NADH.

The complexes share structural similarities being composed of multiple copies of three enzymes: the α-keto dehydrogenase component or E_1 (pyruvate, 2-oxoglutarate or 2-oxo-isovalerate dehydrogenase), the dihydrolipoyl acyltransferase component or E_2, and the dihydrolipoyl dehydrogenase component or E_3, which is an enzyme common to all complexes.

Table 1. Enzymes with Lipoyl or Biotinyl-Bounded Lysine

Enzyme	Substrate	Product	Lysine Position (Accession Number)	Moiety-Bound to Lysine
E_2 component of pyruvate dehydrogenase (human)	pyruvate	acetyl-CoA	K # 100 (P10515)	lipoyl
E_2 component of α-ketoglutarate dehydrogenase (human)	α-ketoglutarate	succinyl-CoA	K # 110 (P36957)	lipoyl
E_2 component of branched chain α-ketoacid dehydrogenase (human)	leucine, isoleucine and valine oxoacids	isovaleryl-CoA	K # 105 (P11182)	lipoyl
E_3 binding protein (protein X, human)	E_3	supramolecular complex	K # 97	lipoyl
Glycine cleavage system (H protein, human)	glycine	CO_2 and NH_4^+	K # 107 (P23434)	lipoyl
α-Propionyl-CoA[a] carboxylase (rat)	propionyl-CoA	(D,L)-methyl-malonyl-CoA	K # 668 (P05165)	biotinyl

[#] As defined in the SWISS-PROT database.

[a] Interestingly a G-X_{15}-K-X_{10}-G structural motif is highly conserved in various mitochondrial enzymes such as α-propionyl-CoA carboxylase, pyruvate carboxylase, acetyl-CoA carboxylase, methyl crotonyl-CoA carboxylase, and the E_2 component of the branched chain α-ketoacid dehydrogenase complex.[11] These enzymes share the common property to possess a biotinylated or a lipoylated lysine residue, respectively, which play a central role for enzyme activity.

In each complex the E_2 component plays a crucial functional and structural role. Multiple copies of E_2 constitute the structural core of the complex to which multiple copies of the E_1 and E_3 components are bound in a peripheral position tighly but noncovalently. Each E_2 protein chain has a pronounced domain and linker structure. Three domains may be identified that are connected by flexible linker regions: (1) the *C*-terminal inner core housing the active site responsible for the acyltransferase activity; (2) the peripheral subunit binding domain responsible, at least in part, for binding E_1 and E_3 together; and (3) the *N*-terminal domain containing the lipoyl group(s).

The lipoyl domains, whose number depends on the species, contain the α-lipoic acid attached in an amide linkage to the ϵ-amino group of a lysine residue and this acts as a ''swinging arm'' ferrying the substrate between the active sites of the complex. In the enzymatic catalysis, E_1, which has thiamine pyrophosphate (TPP) as a prosthetic group, catalyzes first the decarboxylation of the keto-acid to form hydroxyethyl-TPP. Then the E_2 catalyses the reductive acetylation of α-lipoic acid. The lipoyl group of E_2 receives the acylated TPP from E_1 and transfers this group to coenzyme A forming acetylcoenzyme A. During this process, lipoamide is reduced to dihydrolipoamide. E_3 (dihydrolipoyl dehydrogenase) restores the catalytic activity by oxidizing this back to lipoamide through the reduction of NAD^+ to NADH.

In the PDC, the structure of the E_2 core is further complicated by the presence of an additional protein designed protein X or E_3BP. This protein is similar to E_2 and also contains a lipoyl domain, a peripheral subunit-binding domain, and a *C*-terminal domain which, however, does not have acetyltransferase activity. Protein X is required for the proper association of the E_3 component in yeast and mammalian PDC, and its lipoyl group can also participate in the catalytic reaction.

The relative activities of two enzymes (PDC kinase and PDC phosphatase) control PDC activity via a phosphorylation/dephosphorylation cycle. The E_2 component has been reported to be crucial for such regulation. In bovine PDC, it was demonstrated that the PD kinase binds to the E_2 lipoyl domain region, and that selective removal of the lipoyl prosthetic group leads to dissociation of the kinase.

Glycine cleavage system (GCS). The glycine cleavage system is a multienzyme complex that is located only in the hepatic mitochondrial matrix and catalyzes the oxidation of glycine to carbon dioxide and ammonia, forming NADH and (N^5, N^{10})-methylenetetrahydrofolate. The complex consists of four proteins termed P-, T-, L-, and H-protein. α-Lipoic acid is covalently attached to a lysine in the H-protein (Table 1). During the enzymatic catalysis, P-protein, a pyridoxal phosphate-dependent decarboxylase, catalyzes the release of carbon dioxide from glycine and transfers the methylamine moiety to the lipoyl prosthetic group of H-protein. The α-lipoic acid group is reduced during the transfer. T-protein catalyzes the formation of ammonia and the transfer of one-carbon group from the lipoyl residue of H-protein to tetrahydrofolate. L-protein is a lipoamide dehydrogenase that catalyzes the oxidation of the dihydrolipoyl residue of H-protein and the reduction of NAD^+. Thus, the α-lipoic acid prosthetic group of the H-protein interacts with the active sites of three different enzymes in a similar manner as was found in the α-keto-acid dehydrogenase complexes (KADC).

Free α-lipoic acid

Evidence is accumulating that free α-lipoic acid can act at various levels in biochemical pathways. Interaction of α-lipoic acid with various protein systems has been analyzed, and α-lipoic acid has been observed to be a substrate, an inhibitor, or an effector.

α-Lipoic acid as an enzyme substrate. Both the E_2 and E_3 components of the KADC have been reported to use free α-lipoic acid as a substrate.[12] In particular, E_3 has been observed to catalyze the reduction of α-lipoic acid to dihydrolipoic acid at the expense of NADH. This reduction is at a rate slower than that of the reverse reaction, and with a marked stereospecificity for the naturally occurring R-enantiomer.[13] In the absence of NAD^+, however, the E_3 component can use free α-lipoic acid as the terminal acceptor of electrons, and catalyze the oxidation of dihydrolipoic acid bound to the E_2 component with concomitant production of free α-lipoic acid.[14]

An effect of free α-lipoic acid on the enzyme activity of the glycine cleavage system has also been reported.[15] Free α-lipoic acid was able to replace the entire H-protein of this complex as an acceptor of the methylamine carbanion (the intermediate that is formed during the conversion of glycine to CO_2, NH_4, and N^5, N^{10}-methylenetetrahydrofolic acid) although with an efficacy 1000-fold lower than that of H-protein containing α-lipoic acid as a prosthetic group.

It has also been observed that serine hydroxymethyltransferase, the enzyme responsible for the transformation of a large number of amino acids to glycine, can catalyze in the presence of free α-lipoic acid, the direct decarboxylation of glycine with subsequent formation of methylamine-lipoate.[16] α-Lipoic acid has been reported to be a substrate for a lipoamide dehydrogenase detected in human serum. In this case, the efficacy of α-lipoic acid as substrate was eightfold lower than that of lipoamide.[17]

The ability of the α-lipoic/dihydrolipoic couple to act as hydrogen donors in the reaction catalyzed by Se–peroxidase has also been studied.[18] In the presence of H_2O_2 as a substrate, dihydrolipoic acid reduced the active site of glutathione peroxidase and of hydroperoxide glutathione peroxidase. The efficacy of dihydrolipoic acid was far lower than that of the natural substrate GSH (11 and 15% of the activity observed with GSH as electron donor substrate for glutathione peroxidase and for hydroperoxide glutathione peroxidase, respectively). On the other hand, α-lipoic acid in millimolar concentrations was observed to inhibit both hydroperoxide glutathione peroxidase or glutathione peroxidase activity, with 10-fold more efficiency for hydroperoxide glutathione peroxidase than for glutathione peroxidase. However, due to the high cellular concentration of GSH (which is near 10 mM in hepatocytes), an interference of supplemented α-lipoic acid in the cellular function of glutathione peroxidase system was excluded by the authors.

Glutathione reductase was also reported to use α-lipoic acid as a substrate,[19] leading to a NADPH-dependent formation of dihydrolipoic acid. For glutathione reductase, purified from bovine intestinal gut or human erythrocytes, a higher kinetic efficiency towards the S-stereoisomer occurred; however, for the yeast enzyme a similar rate of oxidation of α-lipoic acid was measured.[19]

Recently, it was found that thioredoxin reductase from calf thymus, calf liver, human placenta, and rat liver can efficiently reduce α-lipoic acid in a NADPH-dependent reaction.[20] Thioredoxin reductase was found to be 2.5 times faster in reducing α-lipoic acid with NADPH than in catalyzing the oxidation of dihydrolipoic acid to α-lipoic acid.

The activity of the enzymes described above are responsible for the formation of dihydrolipoic acid observed in cells or tissue extracts exposed to free α-lipoic acid.[21–23] The relative contribution of the different enzymes in the cellular reduction of α-lipoic acid is controversial. It has been reported that the activity of dihydrolipoyl dehydrogenase (E_3) in the rat liver alone is sufficient for the reduction of 100–150 μmol α-lipoic acid/min/kg body weight.[21] Indeed, nonglutathione thiols, assumed to be dihydrolipoate, appear rapidly in the effluent of the isolated rat liver perfused with lipoate.[3] On the other hand, data from our laboratory indicates that the pattern of α-lipoic acid reduction is tissue specific: the liver appears to reduce lipoate to an equal extent by both the cytosolic glutathione reductase and the mitochondrial E_3, whereas reduction in the heart occurs almost completely by E_3.[24]

α-Lipoic acid as enzyme inhibitor. An inhibitory effect of α-lipoic acid on the activity of various enzymes has also been observed. α-Lipoic acid (at a dose ranging between 50 and 150 mg/kg) prevents the conversion of xanthine dehydrogenase to xanthine oxidase in rat intestine homogenates (at a level ranging from 40 to 52%) after 20 min of incubation at 10°C. An inhibition of buttermilk xanthine oxidase activity by 50 and 42% was observed in the presence of 3 mM α-lipoic acid or dihydrolipoic acid, respectively.[25] However, contrasting results were obtained in our laboratory, where 3 mM dihydrolipoic acid was shown not to have a significant effect on cow milk xanthine oxidase activity.[26]

The effect of free α-lipoic acid on catalase activity has also been analyzed. Dihydrolipoic acid (but not α-lipoic acid) inhibited the in vitro activity of horse liver crystal-pure catalase.[27] According to the authors, the inhibition of catalase might be responsible for the toxic effect observed in pigeons and rats upon endogenous administration of dihydrolipoic acid or α-lipoic acid. Both compounds were more toxic in pigeons (LD_{50} is 80 mg/kg for both α-lipoic acid and dihydrolipoic acid) than in rats (LD_{50} is 187 mg/kg and 182 mg/kg for α-lipoic acid and dihydrolipoic acid, respectively) probably due to the lower blood level of catalase in birds. However, an analysis performed in our laboratory indicated that a 24-h treatment of Jurkat cells with α-lipoic acid (0.1–2 mM) did not result in any inhibition of the enzymatic activity of catalase (Marcocci, personnal communication).

A contribution of the aldose reductase pathway has been hypothesized in diabetic complications, due to the increase in aldose reductase activity during hyperglycemia.[28] However, aldose reductase inhibitors show a beneficial effect on the development of cataract, nephropathy, and neuropathy in experimental diabetic rodents. A dose of 0.5 mM α-lipoic acid inhibited the hyperglycemia-activated aldose reductase activity observed in rat lens incubated for 20 h in the presence of 100 mM glucose.[29] The metal chelation properties of α-lipoic acid may be responsible for this protection; however, a redox action of α-lipoic acid on the aldose reductase sulfhydril groups cannot be excluded.[30]

High doses of α-lipoic acid also has inhibitory effects on the PDC, leading to lactic acidosis. This inhibitory effect was also observed in suicide attempts with doses ranging from 10 to 40 g of α-lipoic acid. However, 500 to 1000 mg are considered as well-tolerated doses without adverse effect in placebo-controlled studies.[31–33] It was shown that the absolute bioavailability (F_{abs}) of R(+) enantiomers was significantly higher compared to the F_{abs} of the S(−) enantiomers of α-lipoic acid in humans.[34]

Recently it has been observed that chronic administration (15.6 μmol/kg) of lipoic acid to rats resulted in a ~30% decrease in the activity of the biotin-dependent

enzymes pyruvate carboxylase and β-methylcrotonyl-CoA carboxylase in the liver.[140] The chemical structure of lipoic acid is similar to that of biotin, and the authors suggest that lipoic acid can either displace biotin from its binding site at holocarboxylase synthetase, or compete with biotin for transport across the cell membrane. These findings were only observed in chronic administration, because lipoic acid would not affect biotin-dependent carboxylases once biotin is covalently bound to the enzymes.[140] Also, enzyme activities remain normal if biotin is present in the diet;[140] hence, such enzyme effects would presumably not cause pathology in patients. Such is the case in heterozygote individuals for carboxylase deficiencies that have a 50% decrease in enzyme activity but show no symptoms.[140] Interestingly, biotinidase (the enzyme that cleaves biotinyllysine) can cleave lipoyllysine[141] (the protein-bound form of lipoic acid), and vice versa with lipoamidase. Such results has led to the suggestion that lipoamidase and biotinidase are, in fact, the same enzyme in plasma.[141]

α-Lipoic acid as protein modifier: redox dependent and redox independent action. It was reported that dihydrolipoic acid, the structural homologues bis- and tetranor lipoate (with carbon chains being 2 and 4 atoms shorter than lipoate, respectively) and the amide form lipoamide, reduce the disulfide group of purified *Escherischia coli* thioredoxin,[14,35] thus promoting the disulfide reductase activity of this small protein that is a crucial element in the cellular systems for the regulation of enzyme activities by thiol oxidation-reduction control.[36]

A dihydrolipoic acid-dependent activation of the oligomycin-sensitive mitochondrial SH-groups in rat heart mitochondria and mitoplasts was observed, concomitantly, with an activation of ATP-synthase and a decrease of ATPase activities.[37] Furthermore, results have been presented suggesting that the oxidation of some vicinal thiols by α-lipoic acid may underlie the release of Ca^{2+} from rat liver mitochondria exposed to α-lipoic acid.[38] α-Lipoic acid was also reported to modify, via thiol-disulfide exchange, the —SH groups of the NADPH–cytochrome P450 reductase thus causing the loss of the enzyme reducing activity.[39,40] A dihydrolipoic acid-dependent reduction of met-hemoglobin to oxy-myoglobin, as well as of ferryl-myoglobin to oxy-myoglobin, was reported in vitro.[41] In the same study, α-lipoic acid was observed to interact efficiently with ferryl-myoglobin. Depending on the presence of hydrogen peroxide, α-lipoic acid may either reduce directly the heme iron to form met-myoglobin or may react with a pyrrole ring to form sulfhydryl-myoglobin.

An in vitro effect of α-lipoic acid on structural modifications induced by glucose on bovine serum albumin (BSA) and lysozyme, has also been documented.[42] Dihydrolipoic acid inhibited BSA glycation, but the α-lipoic acid-derivative tetranorlipoate did not.[43] The protective effect of α-lipoate in this system was suggested to be due to a hydrophobic binding of lipoate near the glycation sites of BSA. For the binding of α-lipoic acid to BSA, a binding ratio of 6 mol α-lipoic acid per mol BSA and a formation constant of $8.7 \times 104 \times M^{-1}$ was calculated. Under the same experimental conditions, α-lipoic acid did not inhibit glycation of low density lipoproteins.

An α-lipoic acid-dependent decrease in the oxidative formation of Nα-(carboxymethyl)lysine (a major product of the oxidative modification of glycated proteins) was recently demonstrated in human serum albumin.[44] Moreover, α-lipoic acid counteracted the decrease of red cell membrane fluidity and the reactivity of protein —SH groups due to hyperglycemic conditions.[45]

Table 2. Summary of the Antioxidant and Metabolic Effects of the α-Lipoate/Dihydrolipoate Couple In Vitro

Action	Effect	Reference
Involvement of lipoate in scavenging reactive oxygen species	OH scavenging	50, 51, 52
	$O_2^{\cdot -}$ scavenging	26, 49
	$^1O_2$ scavenging	70
	ROO scavenging	57
	HOCl scavenging	47, 48
	metal chelation	61, 62
Interactions of lipoate with other antioxidants	reduction of GSSG	58
	vitamin C/E recycling	52, 56, 57
	ubiquinol recycling	59
	thioredoxin reduction	71
	NADH/NADPH	57
Effects of supplemented lipoate on metabolic processes	increased NAD^+/NADH ratio	72
	increased intracellular GSH	67
	stabilization of oxidant-induced increase in intracellular Ca^{2+}	73
	inhibition of NF-κB activation	74

Activity of the α-lipoate as scavenger of free radical species (Table 2). The dithiol nature of lipoate renders this compound highly reactive against a number of reactive oxygen species, and it also has the ability to regenerate oxidized antioxidants. Interest in the antioxidant properties of lipoate originated from the studies of Rosenburg and Culik,[46] who noticed that the administration of α-lipoic acid prevented the symptoms of both vitamin C and E deficiency in guinea pigs and vitamin E deficiency in rats.

Various reaction pathways result in the formation of free radicals. The respiratory burst of neutrophils in response to inflammatory stimuli produces highly reactive oxygen species. Superoxide is formed, which in the presence of superoxide dismutase, forms H_2O_2. This can

Table 3. Use of α-Lipoic Acid in Clinical Trials Involving Hepatic or Hepatic-Related Diseases

Disease	α-Lipoic acid dose	Endpoint	Reference, year
Type II diabetes	1000 mg i.v., 1 d	increased glucose disposal	33, 1996
Type II diabetes	500 mg i.v., 10 d	increased glucose disposal increased insulin-sensitivity index	82, 1996
Diabetic neuropathy	200 mg/d 3 weeks	alleviation of pain	83, 1980
Diabetic neuropathy	600 mg/d, 3 weeks 300 mg/d 10 weeks	decrease of blood malondialdehyde level and decrease of albuminuria	84, 1993
Diabetic neuropathy	600 mg/d 15 weeks	alleviation of pain and paresthesia	32, 85, 1993
Hypercholesterolemia	50 mg/d 1 week	decrease of serum cholesterol	88, 1958
Lipids	500 mg/d 10 days	no change in cholesterol, HDL-cholesterol and triglyceride levels	82, 1996
Alcohol clearance	90 mg/d 1 week	no effect on blood clearance	94, 1961
Hepatic cirrhosis	30 mg/d 3 days	no effect on disease progression	111, 1961
Hepatic cirrhosis	300 mg/d 6 months	no effect on disease progression	112, 1982
Mushroom intoxication	400 mg/d one week	regression of hepatomegaly	113, 1972
Radiation in Chernobyl accident	400 mg/d 4 weeks	spontaneous leukocyte chemoluminescence decrease liver function normalization	118, 1993

then be converted to hyperchlorous acid (HOCl) by the action of myeloperoxidase. α-Lipoic acid and dihydrolipoic acid can scavenge both hydrogen peroxide and HOCl,[47,48] while dihydrolipoic acid can scavenge superoxide.[26,49] Hydrogen peroxide may react with transition metals producing highly reactive hydroxyl radicals. Both α-lipoic acid and dihydrolipoic acid have been shown to scavenge hydroxyl radicals in a metal-catalysis system[26] and in a metal-free reaction of ultraviolet irradiation (UVA) induced by decomposition of the aromatic hydroperoxide model compound NP-III.[50,51] During the course of lipid peroxidation, peroxyl radicals are formed that propagate the reaction. Dihydrolipoic acid can scavenge these radicals, formed from both lipophilic and hydrophilic peroxyl radical generators.[52] Therefore, the lipoate couple represents a potent radical scavenging unit.

When antioxidants react with reactive oxygen species, the antioxidant is converted to a form that is no longer able to function, and is said to be consumed. Therefore, this oxidized product needs to be recycled to its native form to function again. Vitamin E, being a potent peroxyl radical scavenger, is the major chain-breaking antioxidant protecting biological membranes from lipid peroxidation.[53] This task appears difficult because there are approximately 1500 phospholipid molecules to 1 molecule of vitamin E; however, membrane oxidation does not naturally occur and vitamin E is not rapidly depleted. This apparent paradox can be explained by vitamin E recycling from circulating antioxidants. A number of antioxidants can recycle vitamin E including vitamin C, ubiquinols and glutathione.[54,55] Dihydrolipoic acid has only a weak interaction with the tocopheroxyl radical, so the major recycling of vitamin E by dihydrolipoic acid occurs via the intermediary recycling of other antioxidants. Electronic spin resonance studies have demonstrated the recycling of the ascorbyl radical by dihydrolipoic acid, which in turn recycles the chromanoxyl radical produced by oxidation. This has been shown in dioleylphosphatidyl liposomes,[52] erythrocyte membranes,[56] and low-density lipoproteins.[57] Dihydrolipoic acid may also recycle vitamin E by reducing oxidized glutathione,[58] which then reduces the vitamin E radicals. There is now evidence that lipoate supplementation increases tissue ubiquinol content,[59] and ubiquinol can also recycle vitamin E.[60] Therefore, there exists a network of antioxidants in which dihydrolipoic acid can interact and replenish to maintain both lipid and aqueous phase antioxidant status.

Effect of α-lipoic acid as metal chelator (Table 2). In addition to the activities described above, α-lipoic acid and dihydrolipoic acid can chelate a number of metal ions including Cu^{2+}, Fe^{3+},[61] Mn^{2+}, Zn^{2+}, and Cd^{2+}.[62] Chelation of Cu^{2+} by α-lipoic acid was reported to (1) inhibit the Cu^{2+}-catalyzed oxidations in vitro, (2) assist the partition of Cu^{2+} into *n*-octanol, and (3) inhibit the flux of H_2O_2 induced within erythrocytes by exposure to ascorbic acid.[63] It has been shown in vitro that dihydrolipoic acid as well as dihydrolipoamide can remove iron stored inside ferritin by complexing it in the ferric form. The rate of the reaction increased as a function of the ratio of dihydrolipoic acid/ferritin, was unaffected by the iron content of the ferritin itself, and was pH dependent.[64]

Effect of α-lipoic acid on glutathione metabolism. Modulation of cellular glutathione status has long been discussed as a potential therapeutic strategy, taking into account the role of reduced glutathione in a variety of detoxification reactions against oxidizing species, produced during the metabolism of xenobiotics, as well as its involvement in the formation of conjugates with electrophilic metabolites.[62,65]

The influence of α-lipoic acid on the cellular status of glutathione was investigated in various in vitro and in vivo systems. In accordance with the lower redox potential of the α-lipoic acid/dihydrolipoic acid couple, with respect to the GSH/GSSG couple, dihydrolipoic acid is a powerful reductant for GSSG.[66] Moreover, in various cellular systems (human T-lymphocytes cell lines, C6 glial, NB41A3 neuroblastoma, Jurkat cells, Wurzburg cells, human erythrocytes, and peripheral blood lymphocytes) α-lipoic acid treatment (10–100 μM) induced an increase in the cellular level of GSH by 30–70%.[22,67,68] In the Jurkat cell line, the α-lipoic acid dependent increase in cellular glutathione levels was proposed to be due to improved cysteine utilization. The authors suggest that dihydrolipoic acid (formed via cellular α-lipoic acid reduction) reduced extracellular cystine to cysteine, which is transported to the cells more efficiently than cystine, and is promptly used as sources for glutathione synthesis.[67]

An increase in the tissue levels of glutathione in the lung, liver, and kidney, concomitantly with an improvement in the survival after whole-body irradiation, was observed in rats after 11 d of an i.p. treatment of α-lipoic acid at doses of 4.8–16 mg/kg/d.[68] An increase in total glutathione levels in the liver and blood, but not in the kidney, heart, and skeletal muscles of rats, occurred after intragastric supplementation with lipoate (150 mg/kg for 8 weeks). Lipoate-supplemented rats also had lower levels of thiobarbituric reactive species in the liver, heart, and red gastronemius muscle.[69]

α-LIPOIC ACID AS A METABOLIC REGULATOR

Soon after the discovery of the role of α-lipoic acid as an enzyme cofactor in oxidative metabolism, various studies were performed to address its effects on carbohydrate and lipid metabolism.

Effect of α-lipoic acid on carbohydrate metabolism

Data from early studies indicate that α-lipoic acid can improve carbohydrate metabolism, probably acting at various levels of metabolic pathways. Addition of α-lipoic acid (40 μg) to liver slices (45–50 mg) was reported to: (1) increase the incorporation of [^{14}C] from [^{14}C]-glucose into expired CO_2, (2) have little effect on the incorporation from [^{14}C]-2-pyruvate, and (3) decrease the incorporation from [^{14}C]-1-acetate.[75]

Treatment of guinea pigs (250 g weight) with α-lipoic acid (0.5 mg) for 10 d substantially increased the level of lactic acid (30% increase with respect to control values), and decreased the level of citric acid (60% decrease compared to control).[76] These data have been interpreted as α-lipoic acid stimulating the anaerobic conversion of pyruvic acid to lactic acid, a reaction that other authors have indicated to occur in both directions.[77,78]

The liver plays an essential role in maintaining and modulating the serum level of glucose (between 80 and 120 mg/100 ml) by glycogenolysis, glycolysis, and gluconeogenesis. But one of the major features in the pathogenesis of type II diabetes is insulin resistance leading to hyperinsulinemia. Recent studies in experimental type I and type II diabetes has provided emerging evidence that α-lipoic acid can facilitate nonoxidative and oxidative glucose metabolism. Singh[79] observed that α-lipoic acid stimulates glucose uptake in perfused hearts of normal and diabetic rats. Haugaard and Haugaard[80] also found an increase in glucose utilization by rat diaphragms in vitro after α-lipoic acid administration. A similar stimulation of glucose transport activity by α-lipoic acid was observed in isolated epitrochlearis muscle from either insulin-sensitive lean or insulin-resistent obese Zucker rats.[135]

A dose-dependent increase in glucose uptake was observed in L6 muscle cells and adipocytes after treatment with α-lipoic acid, due to translocation of Glut-1 and Glut-4 transporters from the intracellular pool to the membrane.[136] In a type I model of diabetes (streptozotocin-diabetic rats), α-lipoic acid treatment increased muscle Glut-4 content.[138] Jacob et al.[81] found that treatment with α-lipoic acid (100 mg/kg for 10 d) improved (1) insulin mediated 2-deoxyglucose uptake, (2) glucose oxidation, (3) glycogen synthesis, and (4) lowered the plasma level of insulin and the plasma level of fatty acids, thus leading to the conclusion that α-lipoic acid is able to facilitate either nonoxidative or oxidative glucose metabolism in animal models. Also, a dose dependent increase in glucose uptake was observed in human T-cell line (Wurzburg cells) treated with α-lipoate.[72]

A recent study has shown the R− enantimer of α-lipoic acid to be the most effective in enhancing insulin-stimulated glucose transport.[137]

In diabetic patients (Table 3) Jacob et al.[33] demonstrated that α-lipoic acid administration (1000 mg IV) enhanced the insulin-stimulated whole body glucose disposal by about 59%. It was hypothesized that α-lipoic acid could act as an antioxidant protecting the sulfhydryls groups of the glucose transporter systems (Glut-1 and Glut-4). Jacob et al.[82] also showed that a 10-d trial of 500 mg of α-lipoic acid in perfusion resulted in a significant increase in insulin-stimulated glucose disposal (30%) and an increase in the insulin-sensitivity index in type II diabetes patients.

One consequence and major complication of diabetic patients is peripheral neuropathy due in part to reduced endoneuronal bioavailability of glucose and the consequent increase in oxidative stress. But hyperglycemia

leads to the glycation of proteins within the endoneural blood vessels. This biochemical process is associated with a reduction of blood flow in the diabetic nerve, which in turn leads to endoneural hypoxia and finally ischemia. It is well established that ischemia leads to elevated levels of free radicals mediating oxidative stress, which in turn is thought to damage the peripheral nerve. As shown by Packer et al.,[1] α-lipoic acid can prevent the glycation of protein, thus leading to a dose-dependent normalization of nerve blood flow in experimental diabetic neuropathy.

This is the basis of supplementation by α-lipoic acid, approved in Germany for the treatment of diabetic neuropathy. In three clinical trials (Table 3), α-lipoic acid was administered daily with doses ranging from 300 to 600 mg for up to 15 weeks.[32,83–85] There was a reduction of clinical symptoms (alleviation of pain, decrease of paresthesia, burning, and numbness) and biological endpoints (reduction of blood malondialdehyde level, reduction of albuminuria, reduction of hydroperoxides) but there was no measurable change in neurophysiologic endpoints as assessed by sensory or motor nerve velocity.

Effect of alpha-lipoic acid on lipid metabolism

The effect of α-lipoic acid on the metabolism of lipids was investigated in rabbits (2.5–3 kg), receiving a diet containing an excess of cholesterol (1 g cholesterol, 9 g of shredded carrots/d for 7 d). Treatment with α-lipoic acid (5 mg/kg intramuscular injection) starting with the diet, limited the diet-dependent increase of lipids in the plasma, liver, and aorta, measured 15 d following the beginning of the experiment. The antilipidemic effect of α-lipoic acid was also observed when α-lipoic acid treatment started at the end of the high cholesterol feeding, although at a lower extent.[86] However, in male Dutch belted rabbits, maintained for 2 months on a diet containing 2% cholesterol and 6% corn oil, the intraperitoneal injection of 1 mg/kg α-lipoic acid did not have any effect on the diet-dependent increase in serum cholesterol and lipoproteins, or on the formation of atherosclerotic plaques in the aorta. However, the weight gain in animals treated with α-lipoic acid was lower than in those not treated.[87] A drop in both total cholesterol and cholesterol esters levels in blood occurred in a series of cardiovascular patients affected by atherosclerosis, which were treated i.m. for 7 d with 50 mg/d of α-lipoic acid[88] (Table 3). Evidence has also been reported that i.v. treatment of patients with α-lipoic acid improves the process of lipid catabolism,[89] as well as inducing an increase in the serum protein content, suggesting an action of α-lipoic acid at a common step in the metabolic process of degradation and synthesis of lipids and proteins.[90] However, α-lipoic acid treatment had no effect on the level of cholesterol, HDL-cholesterol, and triglycerides in diabetic patients.[83]

INVOLVEMENT OF LIPOIC ACID IN HEPATOLOGY AND THERAPEUTIC USE

Clinical trials have been carried out using α-lipoic acid as a therapeutic agent in various disorders involving liver diseases. Also, α-lipoic acid has been widely studied in experimental designs. We will discuss briefly some of these to critically evaluate the results and to reach conclusions as to the effectiveness of α-lipoic acid in such pathologic conditions.

Animal experiments

Numerous experiments were performed using rodents for investigating the putative role of α-lipoic acid in alcohol, solvent, heavy metal intoxications, and radiation exposure. All these toxic agents alter the redox status of organs, in particular the liver.

Ethanol is metabolized to acetate by alcohol dehydrogenase and aldehyde dehydrogenase, using NAD^+. Acetaldehyde and the increased production of NADH and hydrogen ions are believed to be responsible for many of the hepatotoxic effects of ethanol.[91] The hydrogen generation and the associated alteration of redox state produces changes in the flux and metabolism of various hepatic substrates, and this has been associated with the development of lactic acidosis, ketosis, and hyperuricemia.[92] The excess of produced NADH inhibits NAD^+, requiring reactions such as gluconeogenesis, fatty acid oxidation, and xanthine dehydrogenase. Inhibition of the latter results in a shift of purine oxidation to xanthine oxidase and subsequent formation of reactive oxygen species.[93] There is also a significant increase in inductable cytochrome P_{450} 2E1 contributing to lipid peroxidation and subsequent increase of superoxide radicals. In addition, alcohol intoxication leads to a decrease of the hepatic content in GSH as shown in animals and humans.[93]

Akabane et al.[94] studied the effect of α-lipoic acid on the disappearance rate of alcohol and acetaldehyde in rabbit alcohol intoxication. The results in experimental animals receiving 2 mg/kg i.v. α-lipoic acid 15 min before 1 g/kg of alcohol i.v. showed that α-lipoic acid had no influence on blood glucose, lactate, and pyruvate levels, but following an acetaldehyde injection (the metabolic product of alcohol) lactate, α-ketoglutarate, and pyruvate blood concentrations declined and remained significantly lower than those of a control, in a range varying from 10 to 30%. So, in acute alcohol intoxication, the beneficial effect of α-lipoic acid can be ex-

plained by the opposing actions of alcohol and α-lipoic acid on the NADH/NAD^+ ratio: alcohol lowering this ratio, in contrast to α-lipoic acid, which increases this ratio via its cellular reduction to dihydrolipoic acid using NADH and NADPH to produce NAD^+ and $NAPD^+$, allowing reactions such as gluconeogenesis and fatty acid oxidation.[72] However, α-lipoic acid caused a dose-dependent inhibition of gluconeogenesis in rat hepatocytes, which could be abolished by octanoate.[139] This effect was proposed to be due to a sequestration of intramitochondrial coenzyme A.[139]

Halogenated hydrocarbons are lipophilic toxicants readily absorbed after inhalation or ingestion. Like most other organic solvents, halogenated hydrocarbons depress the central nervous system. However, the most serious delayed toxic effects of carbon tetrachloride (CCl_4) are hepatotoxicity and nephrotoxicity. This can be examined by the measurement of serum transaminase levels. Serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase levels (SGOT) were determined during experimental CCl_4 poisoning of rats, with and without α-lipoic acid pretreatment. One experimental CCl_4-poisoning study reported that lipoic acid administration was effective in hepatic function restoration.[95] These studies showed that lipoate pretreatment lowered the levels of SGOT and SGPT significantly.[96] Recently, it was shown that the biotransformation of CCl_4 (1) produces reactive oxygen species by cytochrome P_{450}; and (2) activates calcium channels by increasing the binding of ryanodine, which is a specific ligant of these channels. This activation could be explained by the oxidation of lipophilic thiols, as this effect was partially reverted by α-tocopherol treatment.[97] This fact has to be connected with the finding that α-lipoic acid is able to modulate cytochrome P_{450} reductase. It was shown by Slepneva et al.[39] that α-lipoic acid is able to inhibit both purified and microsomal P_{450} reductase by inducing a chemical modification of the SH-groups via a thiol–disulfide exchange reaction.[39,40] This fact could explain the lower CCl_4 toxicity observed in presence of α-lipoic acid.

The protective affects of α-lipoic acid against free radical-mediated injury interested several groups in examining whether lipoic acid or dihydrolipoic acid protects hematopoietic tissues in mice from free radical damage induced by ionizing radiation. Recently, protective effects of α-lipoic acid against radiation damage were observed by Ramakrishnan et al.[98] They determined the LD_{50} by the endogenous and exogenous spleen colony assay. Intraperitoneal administration of α-lipoic acid at a nontoxic dose of 200 mg/kg body, 30 min before irradiation increased the LD_{50} from 8.67 to 10.97 Gy in male CD2F mice. Dihydrolipoic acid also protects against UVB radiation in mice skin.[99]

Arsenic is found in soil, water, and air as a common environmental toxicant. The trivalent arsenicals are the basic compounds responsible for this toxic action, and of these the aromatics are the most toxic. Arsenic can bind to thiols, and many enzymes are dependent on free thiols for their action. A common example is the pyruvate dehydrogenase complex (PDC) requiring protein-bound α-lipoic acid for the acetylation of CoA. Peters[100] showed that SH-groups present in PDC were a selective target of lewisite and phenyl arsenoxide, as a smaller concentration of As^{3+} was needed to poison these enzymes. As a consequence of that inhibition, pyruvate oxidation was selectively inhibited in vivo and pyruvate accumulated in the blood of poisoned animals. The selective toxicity of arsenicals for PDC was attributed to their affinity and their binding to the α-lipoic acid prosthetic group. In addition, in experiments using dogs, a 17 mg/kg sodium arsenite intoxication was shown to be prevented and reversed by α-lipoic acid.[101] Similarly, a protective action of 2,3-dimercaptoethanol and lipoic acid was observed in a mice viability test by Marino and Reduzzi[102] following a 600 mg/kg of novarsobenzene injection. α-Lipoic acid also strongly inhibited arsenic uptake in rat digestive tracts.[103] These experimental data showed that α-lipoic acid can reverse arsenic-induced enzyme inhibition. If we rate that arsenic is an environmental contaminant in specific populations the effect of lipoic acid could be beneficial in chronic arsenic poisoning treatment.

It has to be noted that α-lipoic acid increases the sulfhydryl content of bile not only by his own reduction by hepatocytes[104] but also by increasing the hepatobiliary transfer of glutathione.[105] This fact is important in considering studies on the effect of α-lipoic acid as a treatment of mercury, lead, and gold poisoning. The results obtained with mercuric chloride intoxicated animals (20 mg/kg) showed that simultaneous administration of α-lipoic acid (177 mg/kg) completely protected mice that had received a lethal dose of mercuric chloride. The number of molar equivalents necessary for protection (8:1) was, however, greater than that observed in the case of arsenite. Similarly, the hepatobiliary transfer of mercuric chloride (10 mmol/kg) was increased in rats (from 12- to 37-fold) following an i.v. administration of 37.5 to 300 mmol/kg of α-lipoic acid. As inorganic mercury is minimally affected by glutathione depletion, Gregus et al.[105] concluded that endogenously reduced dihydrolipoic acid can form stable complexes with mercuric ions, and play the role of an inorganic mercury carrier in bile. In contrast, no effect was shown on the biliary excretion of methylmercury.[104,105] However, although hepatobiliary transport of metals is reported to be glutathione dependent,[106,107] α-lipoic acid did not increase, but rather decreased, the biliary excretion of

methylmercury, cadmium, zinc, and copper.[105] In this case, a temporary formation of dihydrolipoic acid–glutathione mixed disulfide was postulated. The glutathione–α-lipoic acid complex was hypothized to be unable to chelate the metals, and after being translocated into bile was thought to be cleaved to α-lipoic acid and reduced glutathione.

In the case of gold and lead intoxication, it was not possible to protect mice from the lethal effect because of the limit imposed on the use of α-lipoic acid by its own toxicity.[101] Concerning hepatocytes exposed to cadmium (150 mM Cd^{2+}),[108] the addition of α-lipoic acid (1 to 6 mM) or dihydrolipoic acid (17 to 89 mM) resulted in the amelioration of cadmium-induced membrane damage (evidenced by lesser aspartate aminotransferase leakage), the decrease of lipid peroxidation (measured by thiobarbituric acid-reactant) and the depletion of cellular glutathione.

As exemplified by numerous animal studies, the role of α-lipoic acid is either as a metabolic stimulant or an antioxidant displaying a broad range of beneficial effects on chemical and physical agents causing metabolic alterations.

α-Lipoic acid in clinical use

The use of α-lipoic acid in the treatment of alcohol intoxication was reported by Rausch in 1956 and Deutsch in 1960 (cited by Wirtschafter and Smith[109]). They showed that α-lipoic acid was effective in the treatment of hepatic coma and hepatitis, and recommended α-lipoic acid administration during acute alcohol intoxication (Table 3). Shigeta et al.[110] detected lower levels of α-lipoic acid in the sera of cirrhotic patients without determining whether it was due to diet depleted of α-lipoic acid or to the impairment of hepatic functions. This observation led to various trials using α-lipoic acid in cirrhotic patients. The first one, performed on 20 patients treated with 30 mg daily for 3 d, failed to demonstrate any change in serum pyruvate or lactate levels[111] (Table 3). A double-blind trial showed that α-lipoic acid did not influence the course of the disease. Serum aspartate transaminase and γ-glutamyl transpeptidase were only improved in both control and treated patients (300 mg of α-lipoic acid daily for 6 months) following ethanol abstinence.[112] It was reported that in human acute drinking experiments, α-lipoic acid pretreatment (90 mg/d for 7 d) before 540 ml/d of Japanese Sake (equivalent to 1 g/kg of alcohol), had no influence on the blood concentration of alcohol, or acetaldehyde, for a period of 6 h. In humans (in contrast to animals as previously noted), following the ingestion of alcoholic beverages, the blood glucose level roses temporarily, then fell again to the initial values. More interestingly, the blood levels of lactate, pyruvate, and α-ketoglutarate were lower than those seen in the control group without α-lipoic acid.[94]

In recent years the number of cases of mushroom poisoning (mycetism) has increased as a result of the current popularity in the consumption of wild mushrooms. The most serious form of mycetism is produced by *Amanita phalloides* and *Amanita capensis*, which are fairly common in both North America and Europe. These species account for over 90% of all fatal cases. They induce severe and frequent fatal poisoning by virtue of the presence of cyclopeptides (phalloidine, amanitin). The amatoxins (α- and β-amanitin) are a group of cyclic octapeptides that inhibit RNA polymerase II and, hence, block the synthesis of mRNA. α-Lipoic acid has been used successfully in this type of intoxication. Kubicka in 1968 (cited by Zulick et al.[113]) was the first to use α-lipoic acid in this type of mycetism. A significant reduction in hepatorenal damage after administration of α-lipoic acid intravenously was observed. Zulik et al.[113] reported the treatment of 12 patients in Bohemia aged between 16–77 years with transaminases levels over 100 IU/100 ml. They administered α-lipoic acid at 200 mg/daily in two intravenous injections. In these cases hepatomegaly decreased after a week of treatment. The mortality rate was decreased and all patients recovered normal hepatic and renal function. A dose of 300 mg/daily was recommended by Cohen et al.[114] to be given in mushroom poisoning as soon as the serum transaminase levels were raised. It was reported that 39 out of 40 poisoned patients recovered hepatic and renal function in Ceske Budejovice (Bohemia) following this treatment with α-lipoic acid.[115] In the contrary, an analysis of a series of 205 cases of *Amanita* poisoning did not result in any change in the fatality rate following α-lipoic treatment.[116] Based on these facts, the previous author did not recommend treatment with α-lipoic acid, in contrast to Piqueras,[117] who recommended 5 to 10 mg/kg/d in three to four administrations.

Interestingly, a radioprotective effect of α-lipoic acid was observed by Korkina et al.[118] following the Chernobyl accident. Children living in contamined areas (15–40 Ci/km^2) were given either 400 mg daily of α-lipoic acid or the same dose with 200 mg daily of α-tocopherol for 4 weeks. The spontaneous leukocyte chemiluminescence, as assessed by the luminol test, returned to normal levels following 1 month of both treatments. In addition, only the high dosed α-lipoic acid group resulted in a significant decrease in the erythrocyte content of glutathione. It was also mentioned that α-lipoic treatment allowed the normalization of kidney and liver functions.

Lipoamide dehydrogenase deficiency should also be mentioned. This inherited disease affects the activity of PDC, OADC, and BCKADC resulting in blood lactic acidosis, increase of urinary excretion of lactate, 2-keto-

glutarate, and 3-hydroxybutyrate. Dietary supplementation with α-lipoic acid (50 mg/kg/d) over a 2-year period improved the lactic and pyruvic acidosis and the tolerance to protein intake in young patients.[119]

However, it should be noted that only recent trials were correctly conducted using double-blind studies and well-defined end points, especially in lipid and carbohydrate liver metabolism. Moreover, the first human studies involving clinical trials were done with poorly purified and racemic forms of α-lipoic acid. Trials involving more patients in selected liver diseases or liver-related diseases should be performed in the future to better evaluate the exact incidence on lipid and carbohydrate metabolism of α-lipoic acid.

α-Lipoic acid and primary biliary cirrhosis (PBC)

A special mention of the role of α-lipoic acid in hepatology is the role of this cofactor not in the treatment of liver diseases as mentioned in the previous section but as a target for autoantibodies in the rare autoimmune disease, Primary Biliary Cirrhosis.

PBC is an autoimmune disease of the liver occurring mainly in women associated in more than 95% of the patients with antimitochondrial antibodies. This disease is characterized by a destruction of the intrahepatic bile ducts due to the inflammatory response. Antimitochondrial antibodies are directed against a major inner mitochondrial autoantigen consisting of a constituent (E_2) of the pyruvate dehydrogenase complex (PDC-E_2) containing the naturally occurring protein bound lipoic acid.[120]

The antigens. The E_2 component (dihydrolipoyl acetyltransferase) forms the central core of the pyruvate dehydrogenase complex (PDC) and requires protein-bound lipoic acid for its activity. The autoantibodies in PBC are only directed against the E_2 component. This was first shown by Van de Water et al.,[121] who cloned the rat PDH-E_2 and showed that the antigenicity was elicited in a region between amino acids 81 and 100. This residue contains two hydrophilic peaks joined by a hydrophobic region, and also contains the KATIGF (lysine-alanine-threonine-isoleucine-glycine-phenylalanine) motif with lipoic acid covalently bound to the ϵ group of lysine (K). This antigen is often designated as the M_2 (for mitochondrial) antigen.[122]

The other antigens implicated in the immune response are the E_2 component of the branched chain α-keto acid dehydrogenase complex (BCKADC) and the E_2 component of the α-ketoglutarate dehydrogenase complex (KGDC). The E_2 subunits of the PDC the BCKADC and the KGDC both possess an inner lipoyl domain consisting of a lipoylated lysine.[123]

All E_2 components of these 2-ketoacid dehydrogenase multienzyme complexes share some sequence homology. The major epitope is close, if not identical, with the lipoic binding site. Furthermore, two other proteins are also detected by PBC patient's autoantibodies:[124] E_3BP (previously protein X) and the $E_1\alpha$ and $E_1\beta$ subunits of the pyruvate dehydrogenase complex (PDC). E_3BP also contain a lipoylated region (Table 1). It must be noted that the antimitochondrial antibodies display an inhibitory action in vitro against 2-ketoacid dehydrogenase enzymes (KADC).

The antibodies. What is the minimal epitope needed for antibody recognition? Does α-lipoic acid need to be bound to the lipoylated domain for antibody recognition? These are the two central questions to be answered.

Van de Water et al.[121] showed that a 603 bp fragment of the rat dihyrolipoamide acetyltransferase cDNA clone contained all the antigenic reactivity and is recognized specifically by sera of patients with PBC. One synthetic peptide (20 mer) absorbed all serum reactivity to the entire cloned protein. This 20-mer peptide contains the previously discussed KATIGF motif. In the same manner Briand et al.[125] showed that the immunodominant epitope of the E_2 component of the PDC could be mimicked by a synthetic octamer. This synthetic peptide is centered around lysine 173 associated with α-lipoic acid. Interestingly, this approach avoids the use of a carrier molecule by using eight copies of this peptide sequence and allowed the detection of 85% of PBC M^{2+} sera (antimitochondrial type 2 antibodies). This study pointed out that the same multiple peptide construction without α-lipoic acid was unable to react in an ELISA with M^{2+} sera. With PDC-E_2, amino acids 128 to 221 are necessary,[126] but larger regions are important for strong antibody recognition. These studies confirmed that the inner lipoate binding site is a major conformational epitope of E_2 and needs the presence of α-lipoic acid for autoantibody recognition. The importance of the lipoyl moiety was also demonstrated by Fusey et al.,[127] because an octanoyl E_2 PDC was not recognized by patients antimitochondrial antibodies as effectively as the lipoylated counterparts.

Quinn et al.[123] showed that the lipoylated inner domain of E_2 is crucial for the binding and the recognition by the antimitochondrial antibodies of the PBC patients. The patients antibodies directed against the E_2 component of PDC showed higher affinity for the lipoylated form. In contrast, Flannery et al.[129] reported that the presence of α-lipoic acid, or its amide onto the lipoylated domain, is not necessary for displaying antigenicity of the three major antigens. They found other epitopes carried by the $E_1\alpha$ component, a 41 KDa nonlipoylated protein, recognized by 70% sera of PBC patients. The E_2

component of BCKADC and PDC are recognized by different non crossreacting antimitochondrial antibody populations, as shown by immunoadsorption studies, even if 70% of PBC sera react with the E_2 component of BCKADC. For the last enzyme complex, Leung[129] showed that (1) the region spanning residue 1 to 227 (including the α-lipoic acid inner region) is reacting with antimitochondrial antibodies, and (2) that in contrast to the PDC-E_2 component, the antimitochondrial antibodies recognize the recombinant protein even in absence of α-lipoic acid bound to the lipoylated domain.

Pathogenesis. Rowley et al.[130] failed to induce this experimental autoimmune disease by immunizing rats and rabbits with (1) bovine heart, (2) human recombinant and, (3) synthetic peptides of PDC-E_2. The animal antibodies failed also to inhibit the PDC in vitro. The authors' hypothesis was that a critical epitope was not engaged in their experimental paradigm by the immune response. In mice the same fact was demonstrated, even if human recombinant PDC-E_2 induced strong polyclonal and monoclonal antibody response, and even if these antibodies inhibit the function of PDC-E_2, they were unable to reproduce hepatic lesion.[131] But in this study, no experimentally induced antibody was directed against the E_3BP (another α-lipoic acid containing protein of the PDC) or against the outer lipoyl domain, or the mouse PDC-E_2. According to Surh's hypothesis,[131] the autoantibodies are likely the result of a breakdown of tolerance to an unique epitope. A bacterial etiology of PBC has been proposed, as antimitochondrial antibodies recognize both bacterial and mammalian E_2 components.[127]

Moreover, an inhibiting monoclonal antibody (C355.1) raised against PDC-E_2 has been shown by electron microscopy studies to be located around the biliary lumen in the microvilli and the secretory substance of biliary epithelium.[132]

During the last decade the molecular basis of PBC has been clarified; however, the exact pathogenesis of this disease involving lipoic acid remains to be discovered.

At this point, a treatment of PBC could be proposed as the antimitochondrial antibodies are directed against the lipoyl inner motif of PDC. As a synthetic 20-mer[121] or a polymer of an octamer[125] of this motif can react specifically with antimitochondrial antibodies, this disease should benefit from plamapheresis treatment using an affinity column bonded with α-lipoyl synthetic antigens to specifically remove all antimitochondrial antibodies. This should achieve a better clearance of the antimitochondrial antibodies without removing all antibodies, as already carried out using double filtration plasmapheresis, anion exchange, and charcoal adsorption chromatography.[133,134]

CONCLUSIONS

Due to its biochemical characteristics, α-lipoic acid can be considered as a powerful effector of cellular metabolism at different levels and a potential therapeutic agent for the treatment of energy-impaired and redox-unbalanced diseases. A clear pharmacological beneficial effect of α-lipoic acid in diabetes and diabetic-dependent polyneuropathy has emerged from recent studies. For other pathologic conditions further investigation needs to be performed. This is the case in the use of α-lipoic acid in the treatment of hepatic or hepatic-related disorders. Although positive results of α-lipoic acid were observed in animal models, in particular for pathologies related to alcohol assumption, metal, and chemical poisoning, studies in humans often show limitation, discrepancy, and contradiction. Most of the human studies have been done on a limited number of cases, and they lack proper controls. Also, often the dose-dependent effect of the drug has not been evaluated carefully. In this context, recently, it is becoming clear from studies on diabetic neuropathy that beneficial effects of α-lipoic acid may be observed at a relatively high dose, i.e., 600 mg/d. This could explain negative results reported in some previous studies. On the other hand, we have to consider that the liver is the primary organ where α-lipoic acid is metabolized. This can raise the question of the efficiency of the chemical form (redox state, enantiomer, dose), the use of vectors (as cyclodextrins) for better internalization of α-lipoic acid in cells, and should encourage other clinical trials in disorders where oxidative stress, especially in liver diseases, plays a major pathogenic role.

Acknowledgements—This work was in part supported by a grant from the National Institute of Health (DK 50430-01) and from ''Fondation pour la Recherche Medicale'' (SE 000482). We thank Dr. Mulchand S. Patel for critical evaluation of this article. All authors contributed equally to this manuscript.

REFERENCES

1. Packer, L.; Witt, E.; Tritschler, H. J. Alpha-lipoic acid as a biological antioxidant. *Free Radic. Biol. Med.* **19:**227–250; 1995.
2. Podda, M.; Tritschler, H. J.; Ulrich, H.; Packer, L. α-Lipoic acid supplementation prevents symptoms of vitamin E deficiency. *Biochem. Biophys. Res. Commun.* **204:**98–104; 1994.
3. Peinado, J.; Sies, H.; Akerboom, T. P. M. Hepatic lipoate uptake. *Arch. Biochem. Biophys.* **273:**389–395; 1989.
4. Takenouchi, K.; Aso, K.; Kawashima, S. Studies on the metabolism of thioctic acid in skin diseases. *J. Vitaminol.* **8:**99–114; 1962.
5. Gal, E. M.; Razevska, D. E. Studies on the *in vivo* metabolism of lipoic acid. I. The fate of dl-lipoic acid-^{35}S in normal and thiamine deficient rats. *Arch. Biochem. Biophys.* **89:**253–261; 1960.
6. Harrison, E. H.; McCormick, D. B. The metabolism of dl-{1.6-^{14}C} lipoic acid in the rat. *Arch. Biochem. Biophys.* **160:**514–522; 1974.
7. Spence, J. T.; McCormick, D. B. Lipoic acid metabolism in the rat. *Arch. Biochem. Biophys.* **174:**13–19; 1976.

8. Nakamura, T.; Kusunoki, S.; Konishi, S.; Kato, H.; Mibu, A. Studies on the metabolism of lipoic acid in childhood. *J. Vitaminol.* **11:**37–44, 54–59, 60–67; 1965.
9. Jocelyn, P. C. *Biochemistry of the -SH group.* London: Academic Press; 1972.
10. Patel, M. S.; Roche, T. E.; Harris, R. A. Alpha-keto acid dehydrogenase complexes. In: Azzi, A.; Packer, L., eds. *Molecular and cell biology updates.* Berlin: Birkhauser Verlag; 1996:321.
11. Browner, M. F.; Taroni, F.; Sztul, E.; Rosenberg, L. E. Sequence analysis, biogenesis, and mitochondrial import of the α-subunit of rat liver propionyl-CoA carboxylase. *J. Biol. Chem.* **264:**12680–12685; 1989.
12. Reed, L. J.; Koike, M.; Levitch, M. E.; Leach, F. R. Studies on the nature and reactions of protein-bound lipoic acid. *J. Biol. Chem.* **232:**143–158; 1958.
13. Reed, L. J. Studies on the kinetic mechanism of lipoamide dehydrogenase from rat liver mitochondria. *J. Biol. Chem.* **248:**4834–4839; 1973.
14. Bunik, V.; Shoubnikova, A.; Loeffelhardt, S.; Bisswanger, H.; Borbe, H. O.; Follmann, H. Using lipoate enantiomers and thioredoxin to study the mechanism of the 2-oxoacid-dependent dihydrolipoate production by the 2-oxoacid dehydrogenase complexes. *FEBS Lett.* **371:**176–170; 1995.
15. Hiraga, K.; Kikuchi, G. The mitochondrial glycine system. Purification and properties of glycine decarboxylase from chicken liver mitochondria. *J. Biol. Chem.* **255:**11664–11670; 1980.
16. Zieske, L. R.; Davis, L. Decarboxylation of glycine by serine hydroxymethyltransferase in the presence of lipoic acid. *J. Biol. Chem.* **258:**10355–10359; 1983.
17. Pelley, J. W.; Little, G. H.; Linn, T. C.; Hall, F. F. Lipoamide dehydrogenase in serum: A preliminary report. *Clin. Chem.* **22:**275–277; 1976.
18. Maiorino, M. Effect of α-lipoic acid on Se-dependent glutathione peroxidases. In: Packer, L.; Cadenas, E.; eds. *New strategies in prevention and therapy: Biological oxidants and antioxidants.* Stuttgart: Hippokrates Verlag; 1994.
19. Pick, U.; Haramaki, N.; Constantinescu, A.; Handelman, G.; Tritschler, J.; Packer, L. Glutathione reductase and lipoamide dehydrogenase have opposite stereospecificities for α-lipoic acid enantiomers. *Biochem. Biophys. Res. Commun.* **206:**724–730; 1995.
20. Arner, E. S. J.; Nordberg, J.; Holmgren, A. Efficient reduction of lipoamide and lipoic acid by mammalian thioredoxin reductase. *Biochem. Biophys. Res. Commun.* **225:**268–274; 1996.
21. Matuda, S.; Saheki, T. Intracellular distribution and biosynthesis of lipoamide dehydrogenase in rat liver. *J. Biochem.* **91:**553–561; 1982.
22. Han, D.; Tritschler, H. J.; Packer, L. Alpha-lipoic acid increases intracellular glutathione in a human T-lymphocyte Jurkat cell line. *Biochem. Biophys. Res. Commun.* **207:**258–264; 1995.
23. Constantinescu, A.; Pick, U.; Handelman, G. J.; Haramaki, N.; Han, D.; Podda, M.; Tritschler, H. J.; Packer, L. Reduction and transport of lipoic acid by human erythrocytes. *Biochem. Pharmacol.* **50:**253–261; 1995.
24. Haramaki, N.; Han, D.; Handelman, G. J.; Tritschler, H. J.; Packer, L. Cytosolic and mitochondrial systems for NADH–NADPH-dependent reduction of α-lipoic acid. *Free Radic. Biol. Med.* **22:**535–542; 1997.
25. Alvarez, S.; Boveris, A. Inhibition of xanthine oxidase by α-lipoic acid. In: Packer, L.; Cadenas, E., eds. *Biothiols in health and disease.* New York: Marcel Dekker, Inc; 1995.
26. Suzuki, Y. J.; Tsuchia, M.; Packer, L. Thioctic acid and dihydrolipoic acid are novel antioxidants which interact with reactive oxygen species. *Free Radic. Res. Commun.* **15:**255–263; 1991.
27. Cutolo, E. The interaction of 6-8 dithioloctanoic acid with catalase. *It. J. Biochem.* **6:**181–186; 1957.
28. Srivastava, S. K.; Ansari, N. H.; Hair, G. A.; Jaspan, J.; Rao, M.; Das, B. Hyperglycemia-induced activation of human erythrocyte aldose reductase and alteration in kinetic properties. *Biochim. Biophys. Acta* **870:**302–311; 1986.
29. Barnett, P. A.; Gonzalez, R. G.; Chylack, L. T. The effect of oxidation on sorbitol pathway kinetics. *Diabetes* **35:**426–432; 1986.
30. Ou, P.; Nourooz-Zadeh, J.; Tritschler, H. J.; Wolff, S. Ativation of aldose reductase in rat lens and metal-ion chelation by aldose reductase inhibitors and lipoic acid. *Free Radic. Res. Commun.* **25:**337–346; 1996.
31. ASTA Medical Safety Report.
32. Ziegler, D.; Mayer, P.; Muhlen, H.; Gries, F. A. Effeckte einer Therapie mit α-Liponsaure gegenüber Vitamin B1 bei der diabetischen Neuropathie. *Diabetes Stoffw.* **2:**443–448; 1993.
33. Jacob, S.; Clancy, D. E.; Schiemann, A. L.; Simon, I.; Jung, W.-I.; Henriksen, H. J.; Tritschler, H. J.; Augustin, H. J.; Dietze, G. J. Verbesserung der Glucoseverwertung bei Patienten mit Type II—Diabetes mellitus unter α-Liponsaure (Thioctsaure). *Diabetes Stoffw.* **5:**64–70; 1996.
34. Hermann, R.; Niebch, G.; Borbe, H. O.; Fieger-Büschges, H.; Ruus, P.; Nowak, H.; Riethmüller-Winzer, H.; Peukert, M.; Blum, H. Enantioselective pharmacokinetics and bioavailability of different racemic α-lipoic acid formulations in healthy volunteers. *Eur. J. Pharmaceut. Sci.* **4:**167–174; 1996.
35. Holmgren, A. Thioredoxin catalyzes the reduction of insulin disulfides by dithiothreitol and dihydrolipoamide. *J. Biol. Chem.* **254:**9627–9632; 1979.
36. Follmann, H.; Haberlein, I. Thioredoxins: Universal, yet specific thiol-disulfide redox cofactors. *Biofactors* **5:**147–156; 1996.
37. Zimmer, G.; Mainka, L.; Kruger, E. Dihydrolipoic acid activates oligomycin-sensitive thiol group and increases ATP synthesis in mitochondria. *Arch. Biochem. Biophys.* **288:**609–613; 1991.
38. Schweizer, M.; Richter, C. Stimulation of Ca^{+2} release from rat liver mitochondria by the dithiol reagent lipoic acid. *Biochem. Pharmacol.* **52:**1815–1820; 1996.
39. Slepneva, I. A.; Sergeeva, S. V.; Khramtsov, V. V. Reversible inhibition of NADPH-cytochrome P450 reductase by α-lipoic acid. *Biochem. Biophys. Res. Commun.* **214:**1246–1253; 1995.
40. Yelinova, V. I.; Weiner, L. M.; Slepneva, I. A.; Levina, A. S. Reversible modification of cysteine residues of NADH-cytochrome P_{450} reductase. *Biochem. Biophys. Res. Commun.* **193:**1044–1048; 1993.
41. Romero, F. J.; Ordonez, I.; Arduini, A.; Cadenas, E. The reactivity of thiols and disulfides with different redox states of myoglobin. Redox and addition reactions and formation of thiyl radical intermediates. *J. Biol. Chem.* **267:**1680–1688; 1992.
42. Suzuki, J. Y.; Tsuchiya, M.; Packer, L. Lipoate prevents glucose-induced protein modifications. *Free Radic. Res. Commun.* **17:**211–217; 1992.
43. Kawabata, T.; Packer, L. α-Lipoate can protect against glycation of serum albumin, but not low density lipoprotein. *Biochem. Biophys. Res. Commun.* **203:**99–104; 1994.
44. Schleicher, E. D.; Wagner, E.; Nerlich, A. G. Increased accumulation of the glycoxidation product of N^{ϵ}-(carboxymethyl)lysine in human tissues in diabetes and aging. *J. Clin. Invest.* **99:**457–468; 1997.
45. Hofmann, M.; Mainka, P.; Tritschler, H.; Fuchs, J.; Zimmer, G. Decrease of red cell membrane fluidity and -SH groups due to hyperglycemic conditions is counteracted by α-lipoic acid. *Arch. Biochem. Biophys.* **324:**85–92; 1995.
46. Rosenberg, H. R.; Culik, R. Effect of α-lipoic acid on vitamin C and vitamin E deficiencies. *Arch. Biochem. Biophys.* **80:**86–93; 1959.
47. Haenen, G. R.; Bast, A. Scavenging of hypochlorous acid by lipoic acid. *Biochem. Pharmacol.* **42:**2244–2246; 1991.
48. Yan, L.-J.; Traber, M. G.; Kobuchi, H.; Matsugo, S.; Tritschler, H. J.; Packer, L. Efficacy of hypochlorous scavengers in the prevention of protein carbonyl formation. *Arch. Biochem. Biophys.* **327:**330–334; 1996.
49. Suzuki, Y. J.; Tsuchiya, M.; Packer, L. Antioxidant activities of dihydrolipoic acid and its structural homologues. *Free Radic. Res. Commun.* **18:**115–122; 1993.
50. Matsugo, S.; Yan, L.-J.; Han, D.; Tritschler, H. J.; Packer, L. Elucidation of antioxidant activity of alpha-lipoic acid toward

hydroxyl radical. *Biochem. Biophys. Res. Commun.* **208:**161–167; 1995.
51. Matsugo, S.; Yan, L.-J.; Han, D.; Tritschler, H. J.; Packer, L. Elucidation of antioxidant activity of dihydrolipoic acid toward hydroxyl radical using a novel hydroxyl radical generator NP-III. *Biochem. Mol. Biol. Int.* **37:**375–383; 1995.
52. Kagan, V. E.; Shvedova, A.; Serbinova, E.; Khan, S.; Swanson, C.; Powell, R.; Packer, L. Dihydrolipoic acid—A universal antioxidant both in the membrane and in the aqueous phase. *Biochem. Pharmacol.* **44:**1637–1649; 1992.
53. Burton, G. W.; Ingold, K. U. Autoxidation of biological molecules. 1. the antioxidant activity of vitamin E and related chain-breaking phenolic antioxidant in vitro. *J. Am. Chem. Soc.* **103:** 6472–6477; 1981.
54. Packer, L. New horizons in vitamin E research—the vitamin E cycle, biochemistry and clinical applications. In: Ong, A. S. H.; Packer, L., eds. *Lipid-soluble antioxidants: Biochemistry and clinical applications.* Boston: Birkhauser Verlag; 1992:1–16.
55. Sies, H. Strategies of antioxidant defense. *Eur. J. Biochem.* **215:** 213–219; 1993.
56. Constantinescu, A.; Han, D.; Packer, L. Vitamin E recycling in human erythrocyte membranes. *J. Biol. Chem.* **268:**10906–10913; 1993.
57. Kagan, V. E.; Serbinova, E. A.; Forte, T.; Scita, G.; Packer, L. Recycling of vitamin E in human low density lipoproteins. *J. Lipid Res.* **33:**385–397; 1992.
58. Bast, A.; Haenen, G. R. M. M. Regulation of lipid peroxidation by glutathione and lipoic acid: involvement of liver microsomal vitamin E free radical reductase. In: Emerit, I.; Packer, L.; Auclair, C., eds. *Antioxidants in therapy and preventive medicine.* New York: Plenum Press; 1990:111–116.
59. Gotz, M. E.; Dirr, A.; Burger, R.; Wanetzky, B.; Weinmuller, M.; Chan, W. W.; Chen, S. C.; Reichmann, H.; Rausch, W. D.; Riederer, P. Effect of lipoic acid on redox state of coenzyme Q in mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and diethyldithiocarbamate. *Eur. J. Pharmacol.* **266:**291–300; 1994.
60. Kagan, V.; Serbinova, E.; Packer, L. Antioxidant effects of ubiquinones in microsomes and mitochondria are mediated by tocopherol recycling. *Biochem. Biophys. Res. Commun.* **169:**851–857; 1990.
61. Scott, B. C.; Aruoma, O. I.; Evans, P. J.; O'Neill, C.; Van Der Vliet, A.; Cross, C. E.; Tritschler, H.; Halliwell, B. Lipoic and dihydrolipoic acids as antioxidants. A critical evaluation. *Free Radic. Res. Commun.* **20:**119–133; 1994.
62. Sigel, H.; Prijs, B.; McCormick, D. B.; Shih, J. C. H. Stability of binary and ternary complexes of α-lipoate and lipoate derivatives with Mn^{2+}, Cu^{2+}, and Zn^{2+} in solution. *Arch. Biochem. Biophys.* **187:**208–214; 1978.
63. Peimian, O.; Tritschler, H. J.; Wolff, S. P. Thioctic acid (lipoic) acid: A therapeutic metal-chelating antioxidant? *Biochem. Pharmacol.* **50:**123–126; 1995.
64. Bonomi, F.; Pagani, S. Removal of ferritin-bound iron by dl-dihydrolipoate and dl-dihydrolipoamide. *Eur. J. Biochem.* **155:** 295–300; 1986.
65. Meister, A. *Glutathione, chemical, biochemical and medical report.* New York: Wiley-Interscience Publication; 1989.
66. Jocelyn, P. C. The standard redox potential of cysteine-cystine from the thiol-disulphide exchange reaction with glutathione and lipoic acid. *Eur. J. Biochem.* **2:**327–331; 1967.
67. Han, D.; Handelman, G.; Marcocci, L.; Sen, C. K.; Roy, S.; Kobuchi, H.; Tritschler, H. J.; Flohe, L.; Packer, L. Lipoic acid increases de novo synthesis of cellular glutathione by improving cystine utilization. *Biofactors* **9:**1–18; 1997.
68. Busse, E.; Zimmer, G.; Schopohl, B.; Kornhuber, B. Influence of alpha-lipoic acid on intracellular glutathione *in vitro* and *in vivo.* *Arzneimittelforschung* **42:**829–831; 1992.
69. Khanna, S.; Atalay, M.; Laaksonen, D. E.; Gul, M.; Roy, S.; Packer, L.; Hanninen, O.; Sen, C. K. Tissue glutathione homeostasis in response to lipoate supplementation and exercise. Oxygen Club of California, Annual Meeting (February 27–March, 1, 1997), Santa Barbara.
70. Devasagayam, T. P.; Subramanian, M.; Pradham, D. S.; Sies, H. Prevention of singlet oxygen-induced DNA damage by lipoate. *Chem. Biol. Interact.* **86:**79–92; 1993.
71. Gleason, F. K.; Holmgren, A. Thioredoxin and related protein in procaryotes. *FEMS Microbiol. Rev.* **54:**271–297; 1988.
72. Roy, S.; Sen, C. K.; Tritschler, H.; Packer, L. Modulation of cellular reducing equivalent homeostasis by α-lipoic acid: Mechanisms and implication for diabetes and ischemic injury. *Biochem. Pharmacol.* **53:**393–399; 1997.
73. Sen, C. K.; Roy, S.; Han, D.; Packer, L. Regulation of cellular thiols in human lymphocytes by α-lipoic acid: A flow cytometric analysis. *Free Radic. Biol. Med.* **22:**1241–1257; 1997.
74. Suzuki, Y. J.; Mizuno, M.; Packer, L. Signal transduction for NF-κB activation: Proposed location of antioxidant-inhibitable step. *J. Immunol.* **153:**5008–5015; 1994.
75. Bornstein, J.; Hartman, B. D. Effect of thioctic acid on glucose oxidation by liver slices. *Nature* **176:**788–789; 1955.
76. Morgano, G.; Abbona, C.; Alzetta, A.; Sadowski, L. F. Influenza dell'acido tioctico su alcuni metaboliti del fegato. *Arch. E. Maragliano Pat. Clin.* **13:**767–771; 1957.
77. Barker, H. A. Role of lipoic acid in *Butyribacterium rettgeri. Fed. Proc.* **13:**742–743; 1954.
78. Hager, L. P.; Gunsalus, J. C. Lipoic acid dehydrogenase: The function of *Escherischia coli* fraction B. *J. Am. Chem. Soc.* **75:**5767–5768; 1953.
79. Singh, H. P. P. Effect of D,L-alpha lipoic acid on the citrate concentration and phosphofructokinase activity of perfused hearts from normal and diabetic rats. *Biochem. Biophys. Res. Commun.* **41:**555–561; 1970.
80. Haugaard, N.; Haugaard, E. S. Stimulation of glucose utilization by thioctic acid in rat diaphragm incubated in vitro. *Biochim. Biophys. Acta* **222:**583–586; 1970.
81. Jacob, S.; Streeper, R. S.; Fogt, D. L.; Hokama, J. Y.; Tritschler, H. J.; Dietze, G. J.; Henriksen, E. J. The antioxidant α-lipoic acid enhances insulin-stimulated glucose metabolism in insulin-resistant rat skeletal muscle. *Diabetes* **45:**1024–1029; 1996.
82. Jacob, S.; Henriksen, E. J.; Tritschler, H. J.; Augustin, H. J.; Dietze, G. J. Improvement of insulin-stimulated glucose-disposal in type 2 diabetes after repeated parenteral administration of thioctic acid. *Endocrinol. Diabetes* **104:**284–288; 1996.
83. Sachse, G.; Willms, B. Efficacy of thioctic acid in the therapy of peripheral diabetic neuropathy. In: Gries, F. A.; Freund, H. J.; Rabe, F.; Berger, H., eds. *Aspects of autonomic neuropathy in diabetes. Horm. Metab. Res. Suppl.* **9:**105–107; 1980.
84. Kähler, W.; Kuklinski, B.; Ruhlmann, C.; Plotz, C. Diabetes mellitus—a free radical associated disease. Effects of adjuvent supplementation of antioxidants. In: Gries, F. A.; Wessel, K., eds. *The role of anti-oxidants in diabetes mellitus: Oxygen radicals and anti-oxidants in diabetes.* Frankfurt am Main: Verlarg-Gruppe; 1993:33–53.
85. Ziegler, D.; Hanefeld, M.; Ruhnau, K. J.; Meißner, H. P.; Lobish, M.; Schütte, K.; Gries, F. A. Treatment of symptomatic diabetic peripheral neuropathy with the anti-oxidant α-lipoic acid. A 3-week multicentre randomized controlled trial (ALADIN Study). *Diabetologia* **38:**1425–1433; 1995.
86. Angelucci, L.; Mascitelli-Coriandoli, E. Anticholesterol activity of α-lipoic acid. *Nature* **181:**911–912; 1958.
87. Kritchevsky, D. Anticholesterol activity of α-lipoic acid. *Nature* **182:**396–397; 1958.
88. Cattaneo, R.; Levi, E.; Prinotti, C. Acido tioctico e modificazioni di alcuni aspetti del ricambio lipidico in cardioangiopatici aterosclerotici. *Acta Gerontol.* **8:**123–140; 1958.
89. Consolo, F.; Gallone, P.; Janni, A. Acido tioctico e metabolismo lipidico. *Bull. Soc. Ital. Biol. Sper.* **33:**1318–1328; 1957.
90. Gallone, P.; Consolo, F. Acido tioctico e metabolismo protidico. *Bull. Soc. Ital. Biol. Sper.* **33:**1329–1335; 1957.
91. Van Thiel, D. H. Alcoholic liver disease. *Gastroenterology* **81:** 594–615; 1981.

92. Kreisberg, R. A.; Owen, W. C.; Siega, A. M. Ethanol-induced hyperlacidemia: Inhibition of lactate utilization. *J. Clin. Invest.* **50:**166–174; 1971.
93. Lieber, C. S. Role of oxidative stress and antioxidant therapy in alcoholic and nonalcoholic liver diseases. In: Sies, H. ed. *Antioxidants in disease mechanisms and therapy: Advances in pharmacology*, vol. 38. San Diego: Academic Press; 1996;601–628.
94. Akabane, J.; Nakanishi, S.; Kohei, H. Experimental alcohol intoxication and thioctic acid (II). *Med. J. Shinshu Univ.* **6:**13–18; 1961.
95. Paterni, L. Thioctic acid in experimental poisoning with carbon tetrachloride. *Folia Medica (Napoli)* **40:**665–671; 1957.
96. Vancini, B. Glutamic-oxaloacetic and glutamic-pyruvic transaminases in rats subjected to chronic poisoning with carbon tetrachloride. Protective action of thioctic acid. *Arch. Ital. Mal. Appar. Dig.* **25:**352; 1959.
97. Stoyanosvsky, D. A.; Cederbaum, A. I. Thiol oxidation and cytochrome P450-dependent metabolism of CCl_4 triggers Ca^{2+} release from liver microsomes. *Biochemistry* **35:**15839–15845; 1996.
98. Ramakrishnan, N.; Wolfe, W.; Catravas, G. Radioprotection of hematopoietic tissues in mice by lipoic acid. *Radiat. Res.* **130:** 360–365; 1992.
99. Fuchs, J.; Milbradt, R.; Zimmer, G. Dihydrolipoate inhibits reactive oxygen species mediated skin inflammation. *J. Invest. Dermatol.* **94:**526; 1990.
100. Peters, R. A. Biochemistry of some toxic agents I: Present state of knowledge of biochemical lesions induced by trivalent arsenical poisoning. *Bull. Johns Hopkins Hosp.* **97:**1–20; 1955.
101. Grunert, R. R. The effect of dl-α-lipoic acid on heavy-metal intoxication in mice and dogs. *Arch. Biochem. Biophys.* **86:**190–194; 1960.
102. Marino, A.; Reduzzi, F. Thioctic acid and acute intoxication by organic and inorganic arsenicals. *Boll. Soc. Ital. Biol. Sper.* **32:** 368–370; 1956.
103. Tsutsumi, S.; Hattori, K.; Sato, H.; Kawaguchi, M. Studies on the arsenic metabolism. Report 18. On the effects of various antidotes on the enteral absorption of arsenical. *Bull. Tokyo Dent. Coll.* **17:**73–82; 1976.
104. Refsvik, T. Excretion of methylmercury in rat bile: The effect of thiotic acid, thionalide, hexadecyl- and octadecylmercaptoacetate. *Acta Pharmacol. Toxicol.* **50:**196–205; 1982.
105. Gregus, Z.; Stein, A. F.; Varga, F.; Klaassen, C. D. Effect of lipoic acid on biliary excretion of glutathione and metals. *Toxicol. Appl. Pharmacol.* **114:**88–96; 1992.
106. Ballatori, N.; Clarkson, T. Biliary excretion of glutathione and of glutathione–metal complexes. *Fundam. Appl. Toxicol.* **5:**816–831; 1985.
107. Gregus, Z.; Varga, F. Role of glutathione and hepatic glutathione s-transferase in the biliary excretion of methylmercury, cadmium and zinc: A study with enzyme inducers and glutathione depletors. *Acta Pharmacol. Toxicol.* **56:**398–403; 1985.
108. Müller, L.; Menzel, H. Studies on the efficacity of lipoate and dihydrolipoate in the alteration on cadmium toxicity in isolated hepatocytes. *Biochim. Biophys. Acta* **1053:**386–391; 1990.
109. Wirtschafter, Z. T.; Smith, F. W. Effect of lipoic acid on the normal rat liver. *J. Lab. Clin. Med.* **60:**649–654; 1962.
110. Shigeta, K.; Hirauzumi, G.; Wada, M.; Oji, K.; Yoshida, T. Study of the serum level of thioctic acid in patients with various diseases. *J. Vitaminol.* **7:**48–52; 1961.
111. Bravo, M.; Orrego-Matte, H.; Walshe, J. M. Carbohydrate intermediates in hepatic cirrhosis: Role of lipoic acid. *J. Lab. Clin. Med.* **57:**213–222; 1961.
112. Marshall, A. W.; Graul, R. S.; Morgan, M. Y.; Sherlock, S. Treatment of alcohol-related liver disease with thioctic acid. A six month randomized double-blind trial. *Gut* **23:**1088–1093; 1982.
113. Zulik, R.; Bako, F.; Budavari, J. Death-cap poisoning. *Lancet* **2:**228; 1972.
114. Cohen, M. R.; Turco, S.; Davis, N. M. Amanita (phalloides group) mushroom poisoning. Treatment methods including use of thioctic acid. *Drug Intell. Clin. Pharmacol.* **5:**207–209; 1971.
115. Steyn, D. G. The treatment of cases of *Amanita phalloides* and *Amanita capensis* poisoning. *S.A. Med. J.* **40:**405–406; 1966.
116. Floersheim, G. L. Treatment of humanamatoxin mushroom poisoning. Myths and advances in therapy. *Med. Toxicol.* **2:**1–9; 1987.
117. Piqueras, J. Hepatotoxic mushroom poisoning: Diagnosis and management. *Mycopathologia* **105:**99–110; 1989.
118. Korkina, L. G.; Afanas'ef, I. B.; Diplock, A. T. Antioxidant therapy in children affected by irradiation from the chernobyl nuclear accident. *Biochem. Soc. Trans.* **21:**314S; 1993.
119. Matalon, R.; Stumpf, D. A.; Kimberlee, M.; Hart, R.; Parks, J. K.; Goodman, S. I. Lipoamide dehydrogenase deficiency with primary lactic acidosis: Favorable response to treatment with oral lipoic acid. *J. Pediatr.* **104:**65–69; 1984.
120. Gershwin, M. E.; Mackay, I.; Coppel, C.; Nakamura, Y. Clinical Immunologic and molecular features of primary biliary cirrhosis. *Semin. Clin. Immunol.* **7:**5–16; 1994.
121. Van de Water, J.; Gershwin, M. E.; Leung, P.; Ansari, A.; Coppel, R. L. The autoepitope of the 74-kD mitochondrial autoantigen of primary biliary cirrhosis corresponds to the functional site of dihydrolipoamide acetyltransferase. *J. Exp. Med.* **167:**1791–1800; 1988.
122. Tuaillon, N.; Andre, C.; Briand, J.-P.; Penner, E.; Muller, S. A lipoyl synthetic octadecapeptide of dihydrolipamide acetyltransferase specifically recognized by anti-M2 autoantibodies in primary biliary cirrhosis. *J. Immunol.* **148:**445–450; 1992.
123. Quinn, J.; Diamond, A. G.; Palmer, J. M.; Bassendine, M. F.; James, O. F. W.; Yeaman, S. J. Lipoylated and unlipoated domains of human PDC-E2 as autoantigens in primary biliary cirrhosis: Significance of lipoate attachment. *Hepatology* **18:** 1384–1391; 1993.
124. Iwayama, T.; Leung, P. S. C.; Coppel, R. L.; Roche, T. E.; Patel, M. S.; Mizushima, Y.; Nakagawa, T.; Dickson, R.; Gershwin, M. E. Specific reactivity of recombinant human PDC-E1a in primary biliary cirrhosis. *J. Autoimmun.* **4:**769–778; 1991.
125. Briand, J.-P.; Andre, C.; Tuaillon, N.; Herve, L.; Neimark, J.; Muller, S. Multiple autoepitope presentation for specific detection of antibodies in primary biliary cirrhosis. *Hepatology* **16:**1395–1403; 1992.
126. Surh, C. D.; Coppel, R.; Gershwin, M. E. Structural requirement for autoreactivity on human pyruvate dehydrogenase-E2, the major autoantigen of primary biliary cirrhosis: Implication for a conformational autoepitope. *J. Immunol.* **144:**3367–3374; 1990.
127. Fusey, S. P. M.; Ali, S. T.; Guest, J. R.; James, O. F. W.; Bassendine, M. F.; Yeaman, S. J. Reactivity of primary biliary cirrhosis sera with *Escherichia coli* dihydrolipoamide acetyltransferase (E2p): Characterization of the main immunogenic region. *Proc. Natl. Acad. Sci. USA* **87:**3987–3991; 1990.
128. Flannery, G. R.; Burroughs, A. K.; Butler, P.; Chelliah, J.; Hamilton-Miller, J.; Brumfitt, W.; Baum, H. Antimitochondrial antibodies in primary biliary cirrhosis recognize both specific peptides and shared epitopes of the M2 family of antigens. *Hepatology* **10:**370–374; 1989.
129. Leung, P. S. C.; Chuang, D. T.; Wynn, R. M.; Cha, S.; Danner, D. J.; Ansari, A.; Coppel, R. L.; Gershwin, M. E. Autoantibodies to BCOADC-E2 in patients with primary biliary cirrhosis recognize a conformational epitope. *Hepatology* **22:**505–513; 1995.
130. Rowley, M.; Maeda, T.; Mackay, I. R.; Loveland, B. E.; McMullen, G. L.; Tribbick, G.; Bernard, C. C. A. Differing epitope selection of experimentally induced and natural antibodies to a disease-specific autoantigen the E2 subunit of pyruvate dehydrogenase complex PDC-E2. *Int. Immunol.* **4:**1245–1253; 1992.
131. Surh, C. D.; Ahmed-Ansari, A.; Gershwin, M. E. Comparative epitope mapping of murine monoclonal and human autoantibodies to human PDH-E2, the major mitochondrial autoantigen of primary biliary cirrhosis. *J. Immunol.* **144:**2647–2652; 1990.
132. Nakanuma, Y.; Tsuneyama, K.; Kono, N.; Hoso, M.; Van de Water, J.; Gershwin, M. E. Biliary epithelial expression of pyruvate dehydrogenase complex in primary biliary cirrhosis: An immunohistochemical and immunoelectron microscopy study. *Hum. Pathol.* **26:**92–98; 1995.

133. Kogan, A. I.; Findor, J. A.; Igartua, E. B.; Villaravid, N. Intensive plasmapherisis as an alternative therapy for intractable pruritus of primary biliary cirrhosis. *Transfus. Sci.* **12:**197–200; 1991.
134. Yamashita, M.; Sakamoto, H.; Kanao, M.; Shimoyama, T. A long-term double filtration plasmapherisis in the treatment of primary biliary cirrhosis. *Artif. Organs* **17:**143–144; 1993.
135. Henrikson, E. J.; Jacob, S.; Streeper, R. S.; Fogt, D. L.; Hokama, J. Y.; Tritschler, H. J. Stimulation by α-lipoic acid of glucose transport activity in skeletal muscle of lean and obese Zucker rats α-lipoic acid. *Life Sci.* **61:**805–812; 1997.
136. Estrada, E. D.; Ewart, S. H.; Tsakiridis, T.; Volchuk, A.; Ramlal, T.; Tritschler, H. J.; Klip, A. Stimulation of glucose uptake by the natural coenzyme α-lipoic acid/thioctic acid. *Diabetes* **45:**1798–1804; 1996.
137. Streeper, R. S.; Henriksen, E. J.; Jacob, S. J.; Hokama, J. Y.; Fogt, D. L.; Tritschler, H. Differential effects of lipoic acid stereoisomers on glucose metabolism in insulin-resistant skeletal muscle. *Am. J. Physiol.* **273:**E185–E191; 1997.
138. Khamaisi, M.; Potashnik, R.; Tirosh, A.; Demshchak, E.; Rudich, A.; Tritschler, H. J.; Wessel, K.; Bashan, N. Lipoic acid reduces glycemia and increases muscle GLUT4 content in streptozotocin-diabetic rats. *Metabolism* **46:**763–768; 1997.
139. Blumenthal, S. A. Inhibition of gluconeogenesis in rat liver by lipoic acid: Evidence for more than one site of action. *Biochem. J.* **219:**773–780; 1984.
140. Zempleni, J.; Trusty, T. A.; Mock, D. M. Lipoic acid reduces the activities of biotin-dependent carboxylases in rat liver. *J. Nutr.* **127:**1776–1781; 1997.
141. Nilsson, L.; Ronge, E. Lipoamidase and biotinidase deficiency: Evidence that lipoamidase and biotinidase are the same enzyme in human serum. *Eur. J. Clin. Chem. Clin. Biochem.* **30:**119–126; 1992.