of tocopherol deficiency in liver (Bernhard *et al.*, 1963) and in muscle (Witting, 1967). Arachidonic acid, because of its essentiality to the function of cellular membranes, may be closely associated with enzymatically active sites or transport areas and hence be particularly prone to oxidation by both internal and external free radical sources.

Lastly, one should recognize that tocopherol deficiency augmented by oxidant exposure is likely to present multiple foci, in the classical sense. Alterations simultaneously in lipid, protein, sulfhydryl compounds, and nucleotide oxidation states are likely. Serum-reduced glutathione levels are depressed by oxidant exposure but are unaffected by tocopherol intake. Potential interactions may occur since many reactions require sulfhydryl compounds (acyl CoA in desaturases) and since cystine and methionine have been shown to reverse the creatinuria of tocopherol deficiency without alteration of the lipid peroxidation (Desai *et al.*, 1964). In any event, the complexity and, unfortunately, the catastrophic nature of tocopherol deficiency may well preclude human experimentation for some time, but our data suggest that it might be well to recommend the provision of adequate levels of tocopherol for the inhabitants of air-polluted areas.

ACKNOWLEDGMENT

We wish to thank R. M. Danner and J. G. Hadley for their technical assistance. This work was supported in part by P.H.S. Contract 22-68-61.

LITERATURE CITED

Ackman, R. G., Sipos, J. C., *J. Amer. Oil Chem. Soc.* **41,** 377 (1964).
Bernhard, K., Leisinger, S., Pedersen, W., *Helv. Chim. Acta* **46,** 1767 (1963).
Bonner, W. A., *J. Chem. Educ.* **30,** 452 (1953).
Buetler, E., Duron, O., Kelley, B. M., *J. Lab. Clin. Med.* **61,** 882 (1963).
Dam, H., Glavind, J., *Nature (London)* **142,** 1077 (1938).
Desai, I. D., Calvert, C. C. , Scott, M. L., *Arch. Biochem. Biophys.* **108,** 60 (1964).
Draper, H. H., Goodyear, S., Barbee, K. D., Johnson, B. C., *Brit. J. Nutr.* **12,** 89 (1958).
Draper, H. H., Csallany, A. S., *Proc. Soc. Exptl. Biol. Med.* **99,** 739 (1959).
Draper, H. H., *Proc. Soc. Exptl. Biol. Med.* **102,** 737 (1959).
Evans, H. M., Bishop, K. S., *Science* **56,** 650 (1922).
Folch, J., Lees, M., Stanley, G. H. S., *J. Biol. Chem.* **226,** 497 (1957).
Friedman, L., Weiss, W., Wherry, F., Kline, O. L., *J. Nutr.* **65,** 143 (1958).
Goldstein, D. B., Buckley, R. D., Cardenas, R., Balchum, O. J., *Science* **169,** 605 (1970).
Gray, E. L., Goldberg, S. B., Patton, F. M., *Arch. Ind. Hyg. Occ. Med.* **10,** 423 (1954).
Matill, H. A., Golumbic, C., *J. Nutr.* **23,** 625 (1942).
McCay, P. B., Poyer, J. C., Pfeifer, P. M., May, H. E., *Lipids* **6,** 297 (1971).
Menzel, D. B., *Ann. Rev. Pharmacol.* **10,** 379 (1970).
Menzel, D. B., *Arch. Environ. Health* **23,** 149 (1971a).
Menzel, D. B., unpublished data, 1971b.
Mercuri, O., Peluffo, R. O., Bremmer, R. R., *Biochim. Biophys. Acta* **116,** 409 (1966)
Mittler, S., Hedrick, D., King, M., Gaynor, A., *Ind. Med. Surg.* **25,** 301 (1954).
Niehaus, W. G., Jr., Samuelson, B., *Eur. J. Biochem.* **6,** 126 (1968).
Roehm, J. N., Hadley, J. G., Menzel, D. B., *Arch. Environ. Health* **23,** 142 (1971a).
Roehm, J. N., Hadley, J. G., Menzel, D. B., *Arch. Intern. Med.* **128,** 88 (1971b).
Saltzman, B. E., Gilbert, N., *Ann. Chem.* **31,** 1914 (1959).
Schwarz, K., *Z. Physiol. Chem.* **281,** 109 (1944).
Schwarz, K., Bieri, J. G., Briggs, G. M., Scott, M. L., *Proc. Soc. Exp. Biol. Med.* **95,** 621 (1957).
Sedlak, J., Lindsay, R. H., *Anal. Biochem.* **25,** 192 (1968).
Shaw, A. M., Menzel, D. B., Brooksby, G. A., Leon, H. A., *J. Nutr.* submitted for publication (1972).
Struijk, C. B., Beerthius, R. K., *Biochim. Biophys. Acta* **116,** 12 (1966).
Witting, L. A., Horwitt, M. K., *J. Nutr.* **82,** 19 (1964).
Witting, L. A., *Lipids* **2,** 109 (1967).

*Received for review July 9, 1971. Accepted March 13, 1972. Presented at symposium on Chemical Aspects of Nutrition Needs, Division of Agricultural and Food Chemistry, 161st ACS Meeting, Los Angeles, Calif., March–April 1971.*

# Protective Effect of Vitamin E on Plasma Lipid Dienes in Man

Nicholas R. Di Luzio

Since previous studies indicated that lipid peroxidation may be the molecular basis of experimental ethanol-induced hepatic injury, studies were undertaken to determine if diene conjugation could be detected in plasma lipids in conditions of acute and chronic alcoholism. Plasma lipids of human subjects, both normal and diseased, revealed the presence of conjugated diene absorption patterns. The presence of conjugated dienes in plasma lipids, particularly in the phospholipid fraction, may well result from *in vivo* peroxidative events since the administration of lipid antioxidant, as mixed tocopherols, was associated with a significant reduction in plasma lipid conjugated diene levels. This reduction was associated with a significant enhancement in plasma lipid soluble antioxidant activity. Conversely, removal of supplemental vitamin E was associated with a fall in plasma lipid antioxidant activity and a rise in conjugated diene levels, suggesting that the presence of the abnormal conjugated dienes in plasma lipids might be due to a relative antioxidant deficient state or antioxidant imbalance related to excessive polyunsaturated fat intake.

Previous studies from this laboratory have led to the hypothesis that the mechanism of hepatic cell injury, after the administration of such agents as ethanol or carbon tetrachloride, was possibly due to an enhanced peroxidation of lipids (Di Luzio, 1964; Di Luzio and Costales, 1965; Di Luzio, 1966; Comporti *et al.*, 1967; Di Luzio, 1967; Di Luzio and Poggi, 1967; Di Luzio and Hartman, 1967; Di Luzio and Hartman, 1969b). The induced lipid peroxidation was postulated to be related to an ethanol-induced free radical attack on unsaturated lipids of specific hepatic subcellular organelles due to a decreased lipid soluble antioxidant level (Di Luzio and Hartman, 1969a). The protection of animals from the acute or chronic effects of such hepatotoxic agents as ethanol and carbon tetrachloride by the administration of antioxidants was proposed to be caused by an inhibition of lipid peroxidation (Di Luzio, 1964; Di Luzio and Costales, 1965; Di Luzio, 1966; Comporti *et al.*, 1967; Di Luzio, 1967;

Department of Physiology, Tulane University School of Medicine, New Orleans, Louisiana 70112.

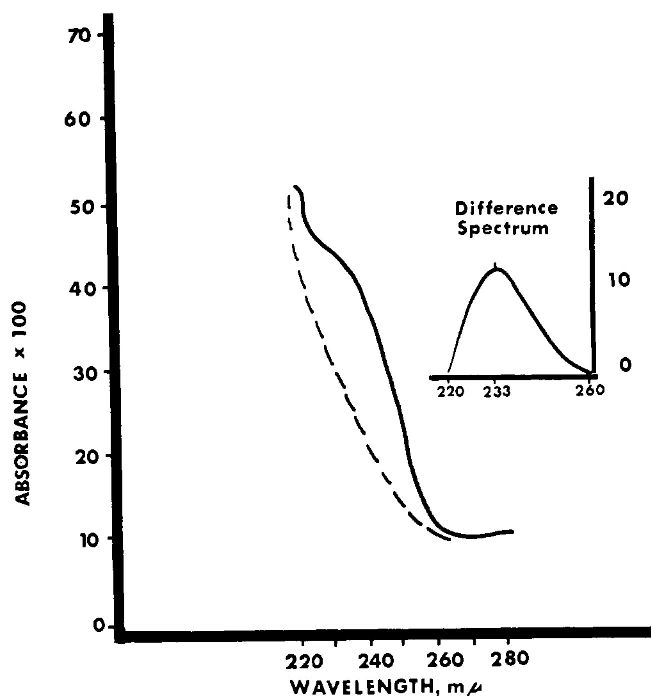


Figure 1. Diene conjugation absorption of plasma total lipids of two normal subjects. Lipid concentration 1 mg/ml of heptane. A typical difference spectrum characteristic of lipid conjugated dienes is manifested in one subject (—), which typified the population studied and is compared to the absorption spectrum of one of the three individuals (- -) which did not manifest the typical conjugated diene spectrum

Di Luzio and Hartman, 1967; Hartman and Di Luzio, 1968; Di Luzio and Hartman, 1969a,b; Hartman *et al.*, 1969). The role of lipid peroxidation in carbon tetrachloride hepatotoxicity has been reviewed by Recknagel (1967).

Since experimental evidence suggested that lipid peroxidation might play a role in experimental ethanol-induced hepatic injury, studies were undertaken to determine if conjugated dienes could be detected in states of acute or chronic alcoholism in man. Conjugated diene analysis (Bolland and Koch, 1945) has been employed as a quantitative estimation of lipoperoxidation (Recknagel and Ghoshal, 1966; Goshal *et al.*, 1969; Di Luzio, 1968; Di Luzio and Hartman, 1969a,b) in a variety of experimental conditions. The hydroperoxide value of autoxidized methyl ester unsaturated fatty acids also has been demonstrated to parallel the conjugated diene value (Frankel, 1962).

The detection of conjugated dienes in the plasma lipid fraction of individuals with clinical histories of alcoholism as well as in plasma lipid fractions of normal subjects prompted a further investigation of the evaluation of the influence of vitamin E administration on plasma lipid conjugated diene levels in normal subjects.

## METHODS

Plasma was obtained from a group of patients with long histories of alcoholism, as well as normal subjects. In addition, patients with a variety of disease syndromes were also evaluated for plasma conjugated dienes. These included individuals with gastric or duodenal ulcers, pulmonary tuberculosis, pneumonia, varying types of neoplasia, nutritional cirrhosis, infectious and viral hepatitis, and diabetes. The studies on lipid conjugated dienes were conducted over a 2-year period (1968–1970) and no seasonal variation was detected which could be related to environmental alterations.

Table I. Difference Spectra, at 233 mμ, of Plasma Total Lipid Following Administration and Removal of vitamin E

| Subject | Vitamin E +7 days[a] | Vitamin E −7 days[b] |
|---|---|---|
| N.D. | −0.229 | +0.207 |
| C.C. | −0.140 | −0.045 |
| J.P. | −0.225 | +0.190 |
| L.M. | −0.118 | +0.103 |
| J.M. | −0.228 | +0.010 |

[a] Values are the difference between the optical density of the control value, *i.e.*, zero time, and the value 7 days following vitamin E administration. [b] Values are obtained by difference between optical density obtained 7 days following the removal of vitamin E supplementation and the value obtained following vitamin E administration.

In six normal male subjects, plasma lipid conjugated dienes and plasma lipid soluble antioxidant levels were studied following ethanol ingestion in the amount of 0.5 g/kg. Plasma samples were obtained prior to and at 1, 3, and 6 hr following ethanol ingestion.

Plasma lipids were extracted in purified chloroform–methanol (Sperry and Brand, 1955). Chloroform–methanol extracts were dried in a nitrogen atmosphere and chromatoquality heptane was employed to dissolve the lipid residue for ultraviolet absorption studies. The ultraviolet spectra were determined from 340 to 220 mμ with the lipid concentration of 1 mg of total lipid per milliliter of heptane.

In studies relating to the possible mechanisms of induced plasma conjugated dienes, five normal subjects ingested 1 to 1.5 g of mixed tocopherol concentrate per day for a 7-day period while maintaining normal dietary intake. The α-tocopherol intake was 500–750 mg, while the additional tocopherols were β-, α-, and δ-tocopherols. Plasma lipid soluble antioxidant concentrations (Glavind, 1963; Di Luzio and Hartman, 1960a,b) and conjugated diene analyses were conducted on the plasma prior to the administration of vitamin E, 7 days following the E administration, and then 7 days post E ingestion. All plasma samples were analyzed in duplicate.

## RESULTS

The studies involving 66 patients with a wide variety of clinical disease states and 21 normal subjects revealed no evidence of enhanced conjugated diene levels which could be readily associated with either their previous clinical history of alcoholism or other disease entities. The plasma of 84 of the 87 subjects studied revealed the presence of conjugated dienes as denoted by 230–233 adsorption maximum (Figure 1).

The acute administration of ethanol, in the form of bourbon, to normal subjects induced mean blood ethanol levels of 68.4, 30.9, and 5.0 mg% at 1, 3, and 6 hr, respectively. No significant differences in plasma lipid soluble antioxidant levels or conjugated dienes could be demonstrated following acute ethanol ingestion.

In an effort to demonstrate that the 233 absorption which was manifested in plasma total lipids was due to the presence of peroxidized lipid fractions manifesting conjugated diene configurations, the influence of antioxidant administration on plasma conjugated diene levels was ascertained. These data revealed that vitamin E ingestion significantly lowered the 233 absorption maximum in all cases (Table I). The significant decrease ($P < 0.005$) in the 233 absorption maximum was associated with a significant enhancement in lipid soluble

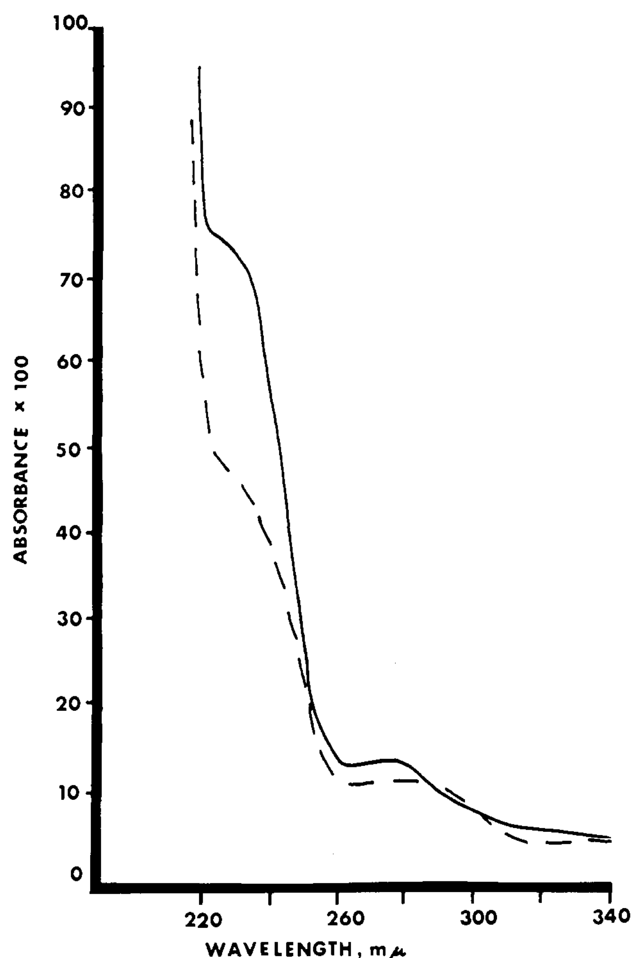


Figure 2. Diene conjugation absorption of plasma total lipids in one subject (N.D.) prior to ingestion of vitamin E (—) and after 1 week of vitamin E administration (- -). The decrease in 233 absorption is readily apparent

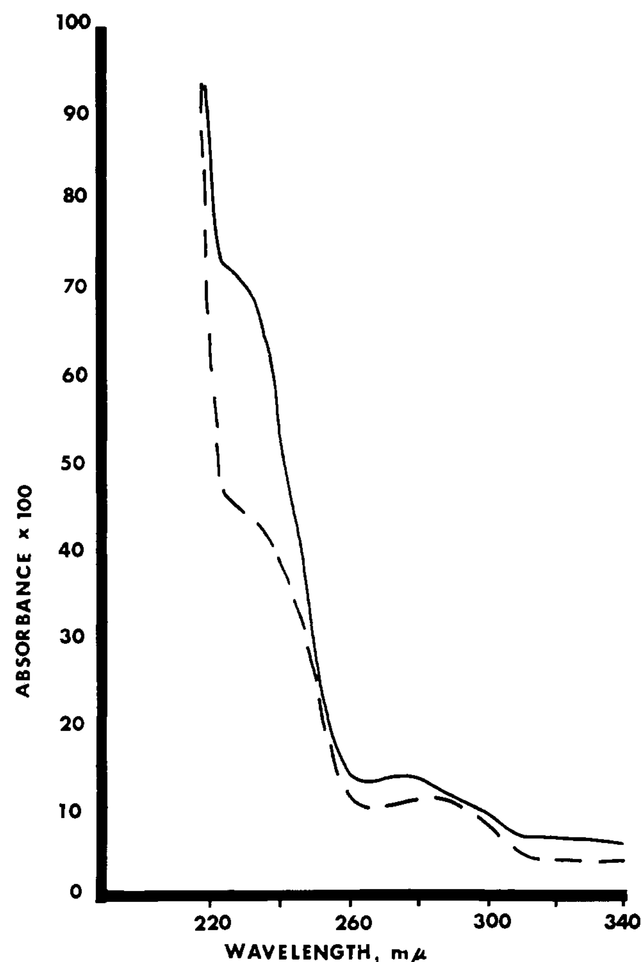


Figure 3. Diene conjugation absorption of plasma total lipids in a subject (N.D.) on high vitamin E intake (- -) and 7 days following vitamin E administration (—). An increase in 233 absorption is obvious following reduction in vitamin E intake

Table II. Influence of vitamin E Administration on Plasma Lipid Soluble Antioxidant Activity[a]

| Subject | Lipid soluble antioxidants, μequiv/l Control | +7 days | −7 days |
|---|---|---|---|
| N.D. | 30.0 | 183.4 | 60.7 |
| C.C. | 39.3 | 106.5 | 43.8 |
| J.P. | 65.0 | 111.2 | 56.1 |
| L.M. | 35.3 | 65.0 | 49.8 |
| J.M. | 109.2 | 144.5 | 73.9 |
| Mean Value | 55.8 | 122.1 | 56.8 |

[a] Vitamin E, as mixed tocopherols, were administered orally in the amount of 1.0–1.5 g/day for 7 days. The α-tocopherol content was 0.5 to 0.75 g/day.

antioxidant activity (Table II). Seven days following the removal of dietary E, a corresponding reduction in lipid soluble antioxidant activity of plasma (Table II) and an elevation ($P < 0.005$) in 233 absorption maximum of plasma lipids (Table I) was observed. Typical responses are denoted in Figures 2 and 3 relative to changes in 233 absorption spectra.

In an attempt to denote the fraction of plasma lipids which contain the conjugated dienes, plasma phospholipids were adsorbed with the aid of Zeolite and the absorption spectra determined on the supernatant which contains triglycerides and free and ester cholesterol (Van Handel and Zilversmit, 1957). The removal of the major components of the absorption spectra when phospholipids were removed from the samples denoted that the conjugated dienes were present in the phospholipids, a fraction characterized by a high level of polyunsaturated lipids (Figure 4).

Since dietary intake of polyunsaturated fatty acids might be an important factor in the elevated conjugated lipids, commercially available oils were purchased and analyzed for the presence of conjugated dienes. The presence of conjugated dienes was pronouncedly manifested in safflower oil (Figure 5), but was not observed in corn oil or peanut oil preparations.

## DISCUSSION

The present study did not demonstrate that circulating plasma lipids of individuals with histories of acute or chronic alcoholism have enhanced levels of plasma lipid peroxides, as denoted by conjugated diene analysis. The studies, however, did demonstrate that normal subjects, as well as those with varying disease states, have measurable conjugated dienes in plasma, which can be significantly reduced when vitamin E ingestion is materially increased. The reduction in conjugated diene levels in the presence of vitamin E ingestion was associated with a significant increase in lipid soluble antioxidant activity in plasma, suggesting an inverse relationship between the level of conjugated dienes and plasma lipid soluble antioxidant activity. This concept was also supported by the finding that 7 days following the removal of supplemental

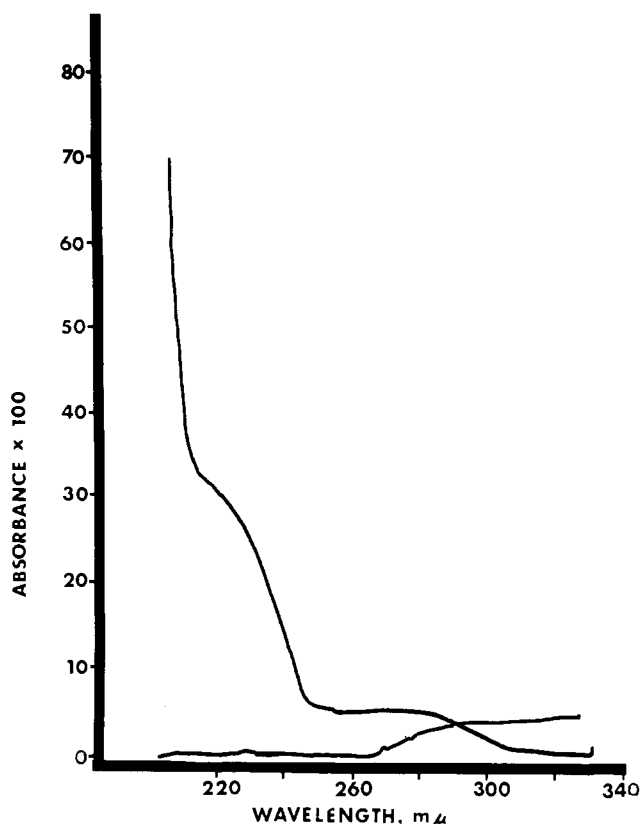


**Figure 4. Diene conjugation absorption of plasma total lipids (upper curve) and the plasma triglyceride fraction (lower curve) of a normal subject. The reduced absorption spectrum indicated that the phospholipid fraction of plasma lipids is the major lipid fraction reflecting diene conjugation**

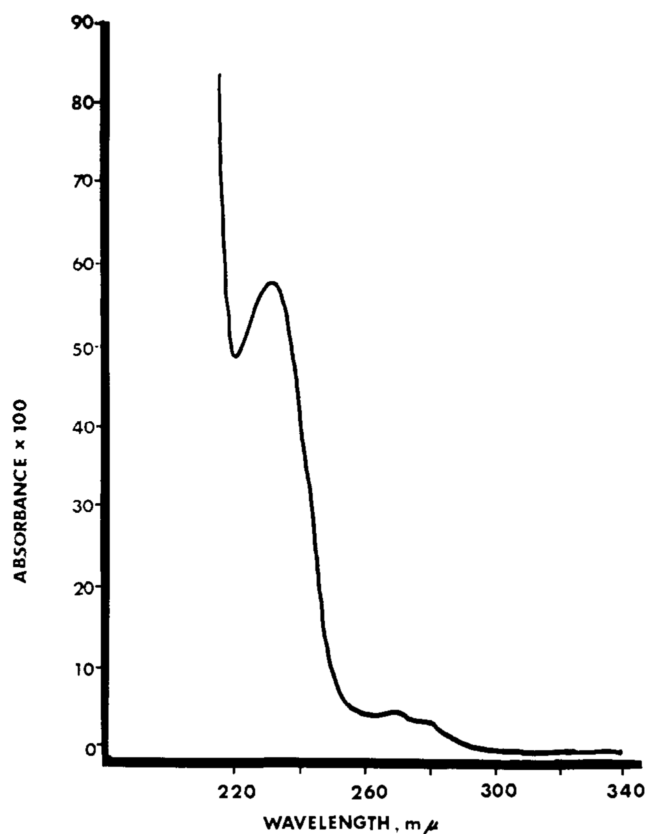


**Figure 5. Diene conjugation absorption of a commercial safflower oil preparation. Lipid concentration 1 mg/ml of heptane. The abnormal conjugated diene absorption is readily detected**

vitamin E, an elevation in conjugated dienes occurred. This finding of rapid loss of vitamin E activity, *i.e.*, within 7 days following removal of vitamin E, denotes a very labile pool of vitamin E in respect to inhibition of plasma lipid diene formation.

The observation that edible oils, such as a commercially available safflower oil, have conjugated dienes present would suggest that the presence of conjugated dienes in plasma lipids might relate to the conjugated diene intake of the individual, due to dienes preexisting in the ingested oils or induced in food preparation. The formation of conjugated dienes could also be due to the relatively low antioxidant level of plasma, *i.e.*, a relative antioxidant deficient state as influenced by the presence of a variety of prooxidants.

The observation of the presence of conjugated dienes in human plasma may well have possible import in human nutrition and disease, if this event reflects the deterioration of lipids, since the injurious effects of free radical induced lipid peroxidation is well established (Tappel, 1965). The plasma conjugated dienes could result from *in vivo* peroxidative events as plasma conjugated diene levels were significantly altered by vitamin E administration. Since conjugated dienes were not observed in plasma of certain other species (Di Luzio, 1971) which are characterized by lower levels of dietary fat intake, as well as polyunsaturated fat intake, a possible dietary contribution may be implied.

Absorption spectra of lung lipids of rats exposed to nitrogen dioxide (Thomas *et al.*, 1968) or lung lipids of mice exposed to ozone (Goldstein *et al.*, 1969) denote the presence of conjugated dienes. Whether the plasma absorption spectra characteristic of conjugated dienes in man relates to environmental influences such as the influence of environmental agents as ozone, nitrogen dioxide or oxygen, also remains to be ascertained.

Clark *et al.* (1969) have reported that lipoproteins from human serum peroxidized *in vitro* with resulting degradation of lipoproteins. The cholesterol ester polyunsaturated fatty acids were found to be selectively degraded. These investigators (Clark *et al.*, 1969) proposed a tentative working hypothesis that *in vivo* peroxidation of lipoproteins may contribute to development of atherosclerosis. The differential oxygen tension on the arterial side, inducing lipid peroxidative events, may be one factor in the phenomenon of arterial lesions. While the present studies denote the presence of conjugated dienes in plasma lipid fractions of man, it is obvious that the source of conjugated dienes in man and their possible implication in disease states clearly remains to be established.

## LITERATURE CITED

Bolland, J. L., Koch, H. P., *J. Chem. Soc.* 445 (1945).
Clark, D. A., Foulds, E. L., Wilson, F. H., *Lipids* **4,** 1 (1969).
Comporti, M., Hartman, A. D., Di Luzio, N. R., *Lab. Invest.* **16,** 616 (1967).
Di Luzio, N. R., *Life Sci.* **3,** 113 (1964).
Di Luzio, N. R., *Lab. Invest.* **15,** 60 (1966).
Di Luzio, N. R., *Progr. Biochem. Pharmacol.* **3,** 325 (1967).
Di Luzio, N. R., *Exp. Mol. Pathol.* **8,** 394 (1968).
Di Luzio, N. R., *Fed. Proc.* in press (1971).
Di Luzio, N. R., Costales, F., *Exp. Mol. Pathol.* **4,** 141 (1965).
Di Luzio, N. R., Hartman, A. D., *Fed. Proc.* **26,** 1436 (1967).
Di Luzio, N. R., Hartman, A. D., "Biochemical and Clinical Aspects of Alcohol Metabolism," V. M. Sardesai, Ed., Charles C Thomas, Springfield, Ill., 1969a, p 133.
Di Luzio, N. R., Hartman, A. D., *Exp. Mol. Pathol.* **11,** 38 (1969b).
Di Luzio, N. R., Poggi, M., "Biochemical Factors in Alcoholism," R. P. Marckel, Ed., Pergamon Press, Oxford, 1967, p 127.

Frankel, E. N., "Symposium on Foods: Lipids and Their Oxidation," H. W. Schultz, E. A. Day, R. O. Sinnhuber, Eds., Avi Publishing Co., Inc., Connecticut, 1962, p 51.
Ghoshal, A. K., Porta, E. A., Hartroft, W. S., *Amer. J. Pathol.* **54,** 275 (1969).
Glavind, F., *Acta Chem. Scand.* **17,** 1635 (1963).
Goldstein, B. D., Ladii, C., Callinson, C., Balchum, O. J., *Arch. Environ. Health* **18,** 631 (1969).
Hartman, A. D., Di Luzio, N. R., *Proc. Soc. Exp. Biol. Med.* **127,** 270 (1968).
Hartman, A. D., Di Luzio, N. R., Trumbull, M. L., *Exp. Mol. Pathol.* **9,** 349 (1969).
Recknagel, R. O., *Pharmacol. Rev.* **19,** 145 (1967).
Recknagel, R. O., Ghoshal, A. K., "Biochemical Pathology," Williams and Eilkins Co., Baltimore, 1966, p 132.
Sperry, W. M., Brand, F. C., *J. Biol. Chem.* **213,** 69 (1955).
Tappel, A. L., *Fed. Proc.* **24,** 73 (1965).
Thomas, H. V., Mueller, P. K., Lyman, R. L., *Science* **159,** 532 (1968).
Van Handel, E., Zilversmit, D. B., *J. Lab. Clin. Med.* **50,** 152 (1957).

*Received for review July 9, 1971. Accepted November 19, 1971. This investigation was supported in part by PHS grant AM-13393 and Hoffmann-La Roche, Inc. Presented at the Division of Agricultural and Food Chemistry, 161st Meeting, ACS, Los Angeles, Calif., March–April 1971.*

# Role of Thiamine Triphosphate in Subacute Necrotizing Encephalomyelopathy

Jack R. Cooper* and Jonathan H. Pincus

Subacute Necrotizing Encephalomyelopathy (SNE) is a fatal, genetic disease in children that, until recently, was only diagnosed postmortem. Since the brain lesions in SNE are very similar to those in Wernicke's Encephalopathy, it had been suggested that SNE represented aberrant thiamine metabolism. We have recently found that extracts of spinal fluid, blood, and urine of patients with SNE inhibit a phosphoryl transferase in brain which catalyzes the synthesis of thiamine triphosphate (TTP) from thiamine pyrophosphate (TPP). This finding was supported by the subsequent observation that the brain of SNE patients at autopsy was essentially devoid of TTP, in contrast to normal brains. This inhibitor in the urine of SNE patients appears to be a protein of molecular weight around 30,000. The presence of this inhibitor in urine has been used successfully to diagnose SNE. Further, we have found that when patients with SNE are treated daily with large doses of either thiamine or thiamine propyl disulfide (a derivative that crosses cell membranes more readily than the vitamin) most patients exhibit marked temporary improvement.

Subacute Necrotizing Encephalomyelopathy (SNE, Leigh's Disease) is a genetic, degenerative disease in childhood which often becomes symptomatic in the first year of life, generally progressing to a fatal termination within a year. Signs of SNE are variable and include feeding problems, weakness, ocular palsy, loss of vision, difficulty in hearing and swallowing, ataxia, and peripheral neuropathy. The constellation of symptomatology is so varied that antemortem diagnosis of this disease is difficult and it has usually been diagnosed only by autopsy. On postmortem examination, lesions in the brain suggest a striking similarity to those seen in Wernicke's Encephalopathy, the classical thiamine deficiency disease. The similarities in clinical and pathological features of both diseases have suggested to clinicians that SNE represents some disorder or abnormality in thiamine metabolism. However, administration of thiamine in moderate amounts to patients with SNE has not resulted in any improvement in their condition. In addition, the enzymes which require thiamine as a coenzyme (pyruvate dehydrogenase, $\alpha$-ketoglutarate dehydrogenase, and transketolase) have all been shown to have normal activity in the brain of a patient who died from SNE (Pincus *et al.*, 1969).

Before going into our studies on the etiology of SNE and possible treatment, it might be worthwhile to review some of the basic aspects of thiamine which we have undertaken in our laboratory.

Department of Pharmacology and Department of Neurology, Yale University School of Medicine, New Haven, Connecticut 06510.

Over the past several years we have been accumulating information which supports our hypothesis that thiamine has a specific role in nervous tissue that is independent of its role as a coenzyme. These studies may be summarized as follows.

1. The electrophysiological effects of pyrithiamine, an antimetabolite of the vitamin, on the isolated rabbit vagus nerve have been shown to be due to a displacement of thiamine from the nerve, rather than to enzyme inhibition (Armett and Cooper, 1965; Cooper, 1968).
2. Thiamine is localized in nerve membranes rather than axoplasm, as shown by fluorescence histochemistry (Tanaka and Cooper, 1968).
3. Both electrical stimulation and neuroactive drugs release thiamine from a variety of intact nerve preparations (Cooper and Pincus, 1967; Itokawa and Cooper, 1970a). Neuroactive drugs also release the vitamin from nerve membrane fractions (Itokawa and Cooper, 1970b).
4. As nerve membranes are purified from a brain homogenate, the percentage of the thiamine triphosphate (TTP) form of the vitamin increases (Itokawa and Cooper, 1970b).
5. Thiamine restores the action potential in an ultraviolet irradiated nerve (Eichenbaum and Cooper, 1971).

There is a suggestion from our work that this neurophysiologically active form of thiamine may be thiamine triphosphate (TTP) in contrast to the coenzyme form, thiamine pyrophosphate (TPP). During the course of these studies we were interested in isolating and characterizing enzymes involved in thiamine metabolism. We isolated and partially purified thiamine pyrophosphatase from the brain (Cooper, 1970) and also isolated two new enzymes, thiamine triphos-