AD-A045 820 CHILDREN'S HOSPITAL MEDICAL CENTER BOSTON MASS F/G 6/1
INTERACTION OF LIPIDS WITH INERT GASES.(U)
MAY 77 H S MICKEL N00014-75-C-0719
UNCLASSIFIED NL

1 OF 1
ADA045820

END
DATE FILMED
11-77
DDC

(12)
B. S.



# INTERACTION OF LIPIDS WITH INERT GASES

N00014-75-C-0719
NR #202-017

## FINAL REPORT

10/1/74 to 2/28/77

Hubert S. Nickel, M.D.
Children's Hospital Medical Center
300 Longwood Avenue
Boston, Massachusetts 02115

Report Date: May 11, 1977





The research was sponsored by the Office of Naval Research under ONR Contract N00014-75-C-0719, NR #202-017.

Hubert S. Nickel, M.D.
Principal Investigator

Joel M. Cohen
Director
Grant and Contract Administration

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

## REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER

2. GOVT ACCESSION NO.

3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle)
INTERACTION OF LIPIDS WITH INERT GASES.

5. TYPE OF REPORT & PERIOD COVERED
FINAL REPORT
10/1/74 to 2/28/77

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s)
Hubert S. Mickel, M.D.

8. CONTRACT OR GRANT NUMBER(s)
N00014-75-C-0719
NR #202-017

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Children's Hospital Medical Center
300 Longwood Avenue
Boston, Massachusetts 02115

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS

11. CONTROLLING OFFICE NAME AND ADDRESS
Office of Naval Research
Department of the Navy
Arlington, Virginia 22217

12. REPORT DATE
May 1977

13. NUMBER OF PAGES
38 + 13 (51)

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)
Final rept.
1 Oct 74 - 28 Feb 77,

15. SECURITY CLASS. (of this report)
Unclassified

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)
Approved for public release: distribution unlimited.
57 pp

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)
Inert Gases
Nitrogen
Lipid peroxidation
Polyunsaturated lipids

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
The role of lipid peroxidation in the induction of disease has been elaborated upon in this report, with examples such as multiple sclerosis and vitamin E deficiency. Autoxidation of the methyl esters of linoleic acid are appreciably different in various oxygen mixture atmospheres, whereas the autoxidation of linoelaidic acid methyl ester is not. This observation has led to a speculation that there is a specific interaction of inert gases with polyunsaturated lipids containing cis bonds.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE
S/N 0102-014-6601

082 790

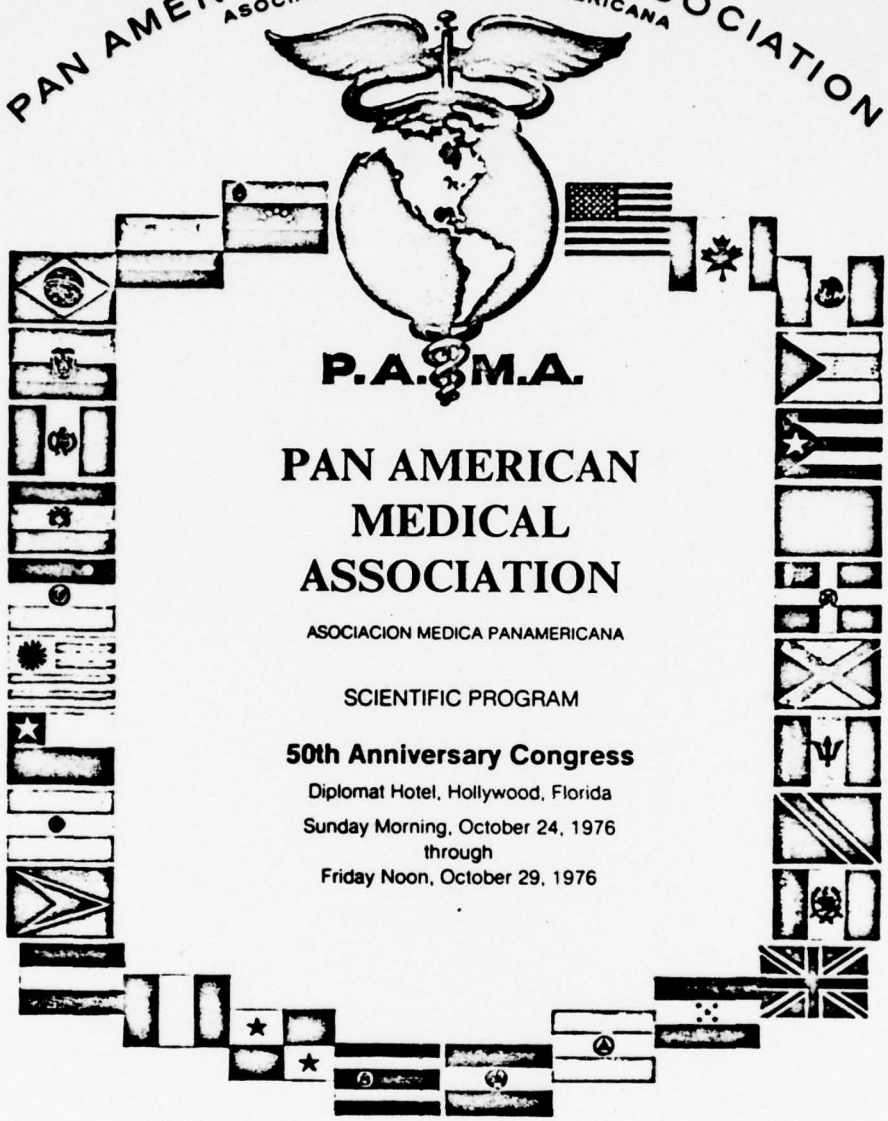
SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

# FINAL REPORT

Contents of Report

Hubert J. Michel MD

PAN AMERICAN MEDICAL ASSOCIATION
ASOCIACION MEDICA PANAMERICANA




# PAN AMERICAN MEDICAL ASSOCIATION

ASOCIACION MEDICA PANAMERICANA

SCIENTIFIC PROGRAM

**50th Anniversary Congress**

Diplomat Hotel, Hollywood, Florida

Sunday Morning, October 24, 1976
through
Friday Noon, October 29, 1976

**Includes**

**DIRECTORY OF 50 MEDICAL SPECIALTY SECTIONS AND OFFICERS (1976-1977)**

# PAN AMERICAN MEDICAL ASSOCIATION INC (PAMA)

ASOCIACION MEDICA PANAMERICANA

EXECUTIVE OFFICES: 745 FIFTH AVENUE, NEW YORK, N.Y. 10022 • TEL. (212) 751-4545



August 24, 1976

Hubert S. Mickel, M.D.
300 Longwood Avenue
Boston, MA 02115

Re: SECTION ON NEUROLOGY

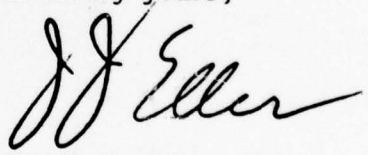
Dear Doctor Mickel:

It is a pleasure to inform you that you have been made a North American Vice President of PAMA's SECTION ON NEUROLOGY. This is in recognition of your stature and contributions to medicine.

We hope that you are planning to attend and/or to participate in PAMA's 50th Anniversary Congress to be held at the Diplomat Hotel, Hollywood, Florida in October 24-29, 1976. If you wish to present a paper, kindly submit the title and a 200-word abstract at your earliest convenience.

With best wishes,

Sincerely yours,



Joseph J. Eller, M.D.
Director General

JJE/jnr
enclosures

## MONDAY, OCTOBER 25, 1976, 9:00 AM–12:30 PM

JOINT MEETING OF:

### Sections on Neurosurgery; and Neurology

*Chairmen:* WILLIAM T. SPENCE, M.D., President, PAMA's Section on Neurosurgery.
LOUIS D. BOSHES, M.D., President, PAMA's Section on Neurology.

**Computerized Brain Scan in Children**
JAMES B. PERRY, M.D., Ft. Lauderdale, Fl.

**Chronic Spinal Arachnoditis, A Neurological Dilemma (Past Experiences and Reflections)**
ALBERT W. AULD, M.D., West Palm Beach, Fl.

**Clinical Microscopic Peripheral Nerve Repair**
HAZEL I. HOLST, M.D., University of Pennsylvania, Philadelphia, Pa.

**Experience With Radiofrequency Facet Rhizotomy in the Treatment of Chronic Neck and Low Back Pain**
JACQUES P. SCHAERER, M.D., Missouri Baptist Hospital, St. Louis, Mo.

**Migraine and Muscular Contraction Headaches, Pathological and Anatomical Considerations**
MASON TRUPP, M.D., Tampa, Fl.

**Third Ventricular Cysticercosis, A Case Report and Review of the Literature**
MICHAEL J. JERVA, M.D., University of Illinois, College of Medicine, Abraham Lincoln School of Medicine, Chicago, Ill.

**Experience With Chymopapain in Protruded Lumbar Disc**
MANUCHER JAVID, M.D., University of Wisconsin Medical Center, Madison, Wis.

**Urea in Neurosurgery – Experience With 2600 Patients**
MANUCHER JAVID, M.D., University of Wisconsin Medical Center, Madison, Wis.

**Central Venous Saturation in Fat Emboli–A New Prognostic Test**
EDWARD M. CORDASCO, M.D. & O. PIEDAD, M.D., State University of Buffalo School of Medicine, Buffalo, N.Y.

**The Use of Fiber-Optic Intracranial Pressure Transducer in Clinical Practice**
ALLAN B. LEVIN, M.D., University of Wisconsin Medical Center, Madison, Wis.

**Midline Echoencephalography**
THOMAS H. MASON, M.D., Schenectady, N.Y.

**Treatment of Diffuse Metastatic Cancer Pain by Stereotaxic Chemical Hypophysectomy**
ALLAN B. LEVIN, M.D., J. KATZ, M.D., University of Wisconsin Medical Center, Madison, Wis.

**Spinal Fusion by Internal Plastic Splint (film)**
WILLIAM T. SPENCE, M.D., Washington, D.C.



## 2:00–4:00 PM (continued)

**Pacinian Corpuscle Neuroma of Digital Nerves**
C. MARTIN RHODE, M.D., Medical College of Georgia, Augusta, Georgia

**Causes of Hand Pain Including the Anatomic Area of the Upper Extremities**
MAX K. NEWMAN, M.D., Southfield, Mich.

**Herpes Simplex Antibodies in Neurologic Diseases**
JOSEPH MENONNA, M.D., P. C. COOK, M.D., and S. COOK, M.D., Fort Lee, N.J.

**Orgasm Headaches**
GEORGE N. LEWIS, M.D., Indiana University Medical School, Bloomington, Ind.

**Sudden Death Due to Colloid Cyst of the Third Ventricle**
GEORGE N. LEWIS, M.D., Indiana University Medical School, Bloomington, Ind.

**A Sampling of Aphasia in Kingston**
JOHN A. A. HALL, M.B., F.R.C.P., Kingston, Jamaica, W.I.

**A New Hypothesis of Multiple Sclerosis**
HUBERT S. MICKEL, M.D., Children's Hospital Medical Center, Boston, Mass.

**On the Illness of Robert Schumann**
HUBERT S. MICKEL, M.D., Children's Hospital Medical Center, Boston, Mass.

**Relief of Headache Pain Without Drugs**
HOWARD D. KURLAND, M.D., Evanston, Ill.

**ECT and ACU-EST in the Treatment of Depression**
HOWARD D. KURLAND, M.D., Evanston, Ill.

## 4:00 PM–5:30 PM (continued)

### ROUND TABLE
### CONVULSIVE DISORDERS

LOUIS D. BOSHES, M.D., Chicago, Ill., moderator

## MONDAY, OCTOBER 25, 1976, 9:00 AM–12:30 PM

JOINT MEETING OF:

### Sections on Rheumatic Diseases and Arthritis; and Geriatrics

*Chairmen:* IRVING WRIGHT, M.D., President, PAMA's Section on Geriatrics
HARRY BARTFELD, M.D., President, PAMA's Section on Rheumatic Diseases & Arthritis.

PAN AMERICAN MEDICAL ASSOCIATION 50TH INTER-AMERICAN CONGRESS

ABSTRACT

# A NEW HYPOTHESIS OF MULTIPLE SCLEROSIS

Hubert S. Mickel, M.D.

Department of Neurology, Children's Hospital Medical Center, Boston, Massachusetts 02115, U.S.A.

Current hypotheses of the pathogenesis of multiple sclerosis center primarily around viral and/or auto-immune etiologies. However, a dietary hypothesis has been proposed by Swank, such that a high-fat diet tends to favor the disease. The anatomical localization of lesions around the post-capillary venules in white matter has been demonstrated by Fog. Alterations in platelet adhesiveness has been found during attacks of multiple sclerosis. Reduced linoleic acid has been noted in both plasma and in areas of white matter not demyelinated, during attacks of recent-onset multiple sclerosis.

This hypothesis implies a role for the peroxide of arachidonic acid, a fatty acid formed from linoleic acid. Arachidonic acid, a major constituent of human platelet phospholipids, when peroxidized, induces platelet aggregation. Peroxidized arachidonic acid is demonstrable in platelets following adsorption of foreign materials on the surface, presumably

zyyvczzczyyvczzcwyyf
tape 30305-cns
fp - 1/15 - lewin - mickel - a - 30305 - X

*International Medical News Service*

HOLLYWOOD, Fla. — The pathogenesis of multiple sclerosis may be an indirect effect of enteric infection, rather than of direct infectin of the glial cells, Dr. Hubert S. Mickel said at the 50th anniversary congress of the Pan American Medical Association.

Sclerotic plaques may be caused by toxic lipid peroxides absorbed or manufactured after enteric infection, reacting with white matter in the nervous system, said Dr. Mickel, of Children's Hospital Medical Center, Boston.

Enteric infection frequently preceeds the onset of multiple sclerosis (MS), and may lead to introduction of chemically reactive lipid peroxides into the body in several ways.

Infection may disrupt the normal barriers against uptake of the peroxides through the intestine and may lead to the conversion of endogenous lipid to peroxide by released lysosomal peroxidases. Endotoxin, which can stimulate peroxidase activity in platelets, may pass more easily through inflamed intestinal mucosa, he said.

Once in the body, lipid peroxides can react with other molecules to create the molecular pathology of MS. Proteins can be denatured or inactivated when lipid peroxides react with their sulfhydryl groups, and the peroxides can attack other polyunsaturated fatty acids, such as linoleic acid.

Patients wiht MS have reduced serum linoleic acid levels. Decreassed linoleate levels appear to increase platelt adhesiveness experimentally, a condition also found in MS victims.

Aggregation of platelets in sites of greatest stasis, postcapilllary venules, may result in the release of peroxidized lipids that could damage endothelial cells and adjuacent oligodendroglia cells. This would explain the perivenular distribution of demyelinating plaques, Dr. Mickel said.

Some investigators have suggested that MS may have an autoimmune etiology. Peroxide-denatured proteins associated with endothelial and oligodenroglia cells could evoke an immune response in the patient.

Chronic MS may be different from the initial attack in that autoimmunity, rather than peroxidative mechanisms, could predominate, he said.

One implication of a peroxide mechanism for MS is that a diet high in polyunsaturated fats and low in antioxidants should have a deleterious effect on the patient. Vegetable fats, although more polyunsaturated than animal fats, contain more antixoidants. Peroxidation is a greater danger with unsaturated fatty acids of animal origin than of plant.

end

Reprinted from PERSPECTIVES IN BIOLOGY AND MEDICINE
Vol. 18, No. 3, Spring 1975

# *MULTIPLE SCLEROSIS: A NEW HYPOTHESIS*

*HUBERT S. MICKEL**

## *Introduction*

"Despite a continuing advance in knowledge concerning many aspects of multiple sclerosis and the existence of several not entirely implausible aetiological theories, a number of experienced workers in this field have professed that they would not be too surprised if the final answer to the questions of causation and pathogenesis in multiple sclerosis involved entirely novel or hitherto unsuspected disease processes" [1].

Multiple sclerosis, as a clinical and pathological entity, was first described by Cruveilhier [2] and Carswell [3]. The term, "sclerose en plaques," appeared in the literature in 1866 in a report by Vulpian, in which two cases were described by Charcot [4]. Charcot called attention to mild cases and *formes frustes*, but further description of the disease —including the triad of nystagmus, scanning speech, and intention tremor—is also attributed to him [5–7].

Many different etiologies have been suggested for multiple sclerosis. A prominent consideration has been an infectious origin of the disease. In 1884, Marie stated that it might be a complication of a number of infectious illnesses [8]. Charcot suggested a relationship to an antecedent illness and cited cases that had followed cholera, smallpox, and typhoid fever. Bullock, in 1913 [9], and Gye, in 1921 [10], claimed to have transmitted multiple sclerosis from man to rabbits, using cerebrospinal fluid injections. In these and other such studies, the failure to identify a specific organism has been a stumbling block. Pette has pointed out the

*Departments of Neurology, Harvard Medical School and Children's Hospital Medical Center, 300 Longwood Avenue, Boston, Massachusetts 02115. Supported by grants from the Office of Naval Research, Department of the Navy, NR 908-108; United Cerebral Palsy Research and Educational Foundation R224-69; National Institutes of Health Developmental Neurology grant NS-HD 09704; National Institutes of Health Children's Hospital Medical Center Mental Retardation and Human Development Research program HD-03773; and National Institutes of Health grant HE-10098. I express my gratitude to the following individuals for their comments and suggestions on reviewing the manuscript prior to its submission for publication: Dr. Charles Barlow, Children's Hospital Medical Center, Boston; Dr. Torben Fog, Kommunehospitalet, Copenhagen, Denmark; and Dr. Floyd Gilles, Children's Hospital Medical Center, Boston. I am also grateful to Lynn Gerrick for her assistance in typing the manuscript.

absence of a preliminary phase in this disease, seen in most viral infections, as well as the predilection of most known neurotropic viral infections for gray matter [11–12]. The possibility of a latent viral infection causing multiple sclerosis is widely considered, as suggested by Gadjusek, Gibbs, and Alpers [13].

The hypothesis that the disease is associated with diffusion of a circulating myelinolytic toxin or enzyme was proposed by Marburg in 1906 [14], and again by Brickner in 1931 [15].

A vascular, thrombogenic etiology of multiple sclerosis was postulated by Putnam in 1933, by the observation of the close proximity of the plaques to small venules in both brain and spinal cord [16–17]. Torben Fog has elaborated on this hypothesis with extensive observations [18].

A dietary etiology of multiple sclerosis was proposed by Swank, who has claimed improvement by treating multiple sclerosis patients with low-fat diets, especially restricted in fats of animal origin [19–20]. Others have shown that there is a decrease in polyunsaturated fatty acids in plasma [21–24] and in brain [25–28] in patients with multiple sclerosis. These observations have been advanced as evidence for a dietary hypothesis for the disease. A diet relatively low in polyunsaturated fatty acids and high in saturated fatty acids is considered to predispose to the disease. The lack of confirmation of these decreases in linoleic acid in plasma and brain in cases of chronic multiple sclerosis has been advanced as evidence against the saturated fatty acid dietary hypothesis [29–30].

An autoimmune etiology is currently also considered possible. The accumulation of lymphocytes and plasma cells in areas of demyelination, as well as the deposition of gamma globulin in the same area, supports this view. However, there is no raised plasma gamma globulin level in multiple sclerosis. On the other hand, cerebrospinal fluid gamma globulin levels are frequently elevated. No correlation has been found between circulating antibodies and the clinical severity of the illness. No clear association exists between multiple sclerosis and known autoimmune diseases. No convincingly consistent therapeutic response occurs to corticosteroid administration. Hence, no more than two of the parameters of immunological disease described by Mackay and Burnet are demonstrated unequivocally in multiple sclerosis [31]. Nevertheless, there has been much interest and investigation in experimental allergic encephalomyelitis as a model for multiple sclerosis.

We are presenting a concept of this disease in which each of these hypotheses of the etiology of multiple sclerosis finds a role.

### *Hypothesis*

The hypothesis of the etiology of multiple sclerosis that we are presenting is based on lipid peroxidation.

Polyunsaturated fatty acids react in an autocatalytic manner with molecular oxygen. The principal primary products of the autoxidation of polyunsaturated fatty acids at ordinary temperatures are hydroperoxides [32–40]. The mechanistic aspects of lipid hydroperoxide formation has been proposed to involve a free radical chain mechanism [41–46]. The net effect of the propagating cycle is a relative decrease in the concentration of polyunsaturated fatty acids due to their consumption in the process [47]. Some antioxidants exert their effect as free radical acceptors and in so doing terminate chain reactions [48].

The very high chemical reactivity of lipid peroxides can result in their denaturation of proteins, by the attack on sulfhydryl groups and other nucleophiles [49–50]. Sulfhydryl groups are frequently associated with reactive sites of enzymes, so that enzymatic activities can be affected by the peroxidative attack of lipid peroxides on proteins [51].

The lipid peroxides that might be implicated in the pathogenesis of multiple sclerosis might arise from the gastrointestinal tract. There appears to be a partial barrier to the absorption of pure lipid peroxides from the intestine [52–53], which might be related, in part, to shedding of damaged mucosal epithelial cells into the intestinal lumen. Autoxidized fats contain hydroperoxides and polymers, but only the peroxides are considered toxic [54]. Autoxidized fats are toxic if fed to animals in sufficient amounts. Degwitz and Lang showed that feeding autoxidized soy bean oil resulted in weight loss and was fatal to young rats [55].

The absorption of lipid peroxides might be markedly affected by enteric inflammation. The epidemiology of multiple sclerosis parallels that of poliomyelitis and suggests an enteric viral infection such as in poliomyelitis to be associated with multiple sclerosis [56]. It might be that any of a number of enteric viruses could produce the postulated derangement of fat absorption. On the other hand, both Charcot and Marie suggested a relationship to antecedent infectious illness, so that it might be that the enteric infection need not be viral. If a number of agents could produce this effect, there would be no specific infectious etiology demonstrable in multiple sclerosis. Epidemiology should parallel that of enteric virus infections in general. In this manner, the relative lack of immunity to enteric viruses in more northerly locations could explain the relative risk of multiple sclerosis that migrating groups have demonstrated [57–60]. Simply stated, if one had had exposure to enteric viruses at an early age, there would be less chance of developing an enteric viral infection during adolescence or adulthood, and, therefore, less chance of developing multiple sclerosis.

However, there are epidemiological data supporting the concept of a latent viral infection in multiple sclerosis, in that individuals, drinking the same contaminated water supply in their youth, developed multiple sclerosis after a comparable "incubation period" [61]. In this case, the proposed primary infection is enteric, and the presumption is that the

infection occurs secondarily in the brain. Could it not be that the latent viral infection occurs in the intestine and that the pathogenetic mechanisms evoked by the virus many years later also be located there?

We propose the following relationship of acute or chronic enteric infections to the absorption of lipid peroxides. The first is that, with an enteric infection, the natural barrier to absorption of lipid peroxides is broken down. The second is that, with an enteric infection, there is enhanced peroxidation of endogenous lipids as a result of the release of lysosomal peroxidases. A third is that, with an enteric infection, there is absorption of materials such as endotoxin, resulting in peroxidation of lipids. A combination of these alternatives is possible. With each of these possibilities, a diet rich in fats and low in antioxidants should be deleterious. Although fats of animal origin are more saturated than fats of vegetable origin, they also contain less natural antioxidants. Unsaturated fatty acids in animal fats might then be more susceptible to peroxidation than those of vegetable origin.

Peroxidase activity is ubiquitous to all mammalian tissues [62]. The neutrophilic granulocyte contains a myeloperoxidase which is involved in the antimicrobial system associated with phagocytosis [63]. Myeloperoxidase is present in the neutrophil in very high concentrations [64–65] and appears to be associated with lysosomes [66–69]. Hydrogen peroxide is formed during the process of phagocytosis [70–74]. Hydrogen peroxide formed might attack polyunsaturated fatty acids. Comparably, there might be a reduction in the cellular content of antioxidants, resulting from the diffusion of hydrogen peroxide. Since peroxidase activity is demonstrated in mammalian tissue, it is not unreasonable to propose enzyme activity within intestinal mucosal cells, or within cells responding to viral or bacterial infection within the intestinal mucosa. Under these circumstances, one might presume that there is altered absorption of lipid peroxides, or even formation of lipid peroxides *de novo* as a result of the inflammatory process in the intestinal mucosa.

Increased absorption of lipid peroxides would initiate further chain reaction propagation, resulting in peroxidative attack on other polyunsaturated fatty acids. Plasma linoleic acid is reported to be decreased in multiple sclerosis [21–24, 75]. That others have not found decreased plasma linoleate in cases of chronic multiple sclerosis, even in exacerbation, suggests that a peroxidative mechanism might not be operative in the chronic form of the disease [29, 76].

A correlation between the decrease in plasma linoleic acid and increase in platelet adhesiveness has been found in multiple sclerosis [77–78]. An alteration in platelet function is an observation reported frequently in multiple sclerosis [79–85]. A decrease in plasma linoleate in multiple sclerosis might be the result of peroxidative consumption of polyunsaturated fatty acids [47]. Altered platelet function might be the result of peroxidized fatty acids adsorbed onto platelet surfaces [86].

It has been demonstrated that the phenomenon of platelet adhesiveness is comparable to platelet aggregation [87]. In elaborating this hypothesis, the altered platelet adhesiveness found in multiple sclerosis is correlated with the finding of enhanced platelet aggregation resulting from peroxidized arachidonic acid. In the presence of peroxidized arachidonic acid, human platelets harvested even 24 hours previously aggregate vigorously and abruptly. Peroxidized arachidonic acid can be recovered from a pellet of platelets, after incubation with the peroxidized lipid in vitro, suggesting that the peroxidized fatty acid can be associated with platelet surfaces without destruction [86]. Lipid peroxides have been recovered from human platelets, increasing with the harvested age of the platelets [88–89].

Platelets have been described as spongelike, since they carry many substances on their surfaces [90–91]. Free fatty acids have been adsorbed onto platelet surfaces [92]. Therefore, we propose that peroxidized arachidonic acid, which enhances platelet aggregation, might be adsorbed onto the surface of platelets in vivo, without the labile peroxyl group being destroyed, since this phenomenon appears to occur during in vitro studies [86].

*Still another possibility whereby* lipid peroxides might occur in association with platelets could be with formation of lipid peroxides by platelet peroxidase [93]. The finding of peroxidase activity is a pronounced histochemical feature of megakaryocyte derivatives [94]. The adsorption of materials such as thrombin, polystyrene-latex particles, or heterologous antibody onto platelets results in the production of increased amounts of lipid peroxides, presumably resulting from platelet peroxidase [95]. It has been shown that endotoxin has a marked affinity for adsorption onto platelets [96–97]. Platelet peroxidase might be activated by the adsorption of endotoxin, which had been absorbed from the intestine as a result of enteric inflammation. With the release and activation of platelet peroxidase, arachidonic acid—present in high concentration in platelet lipids [98]—would be one of the principal lipid constituents peroxidized as a by-product.

Samuelsson has reported the formation of 12-hydroperoxy arachidonic acid from platelet lipoxygenase and the formation of the endoperoxide and 15-hydroperoxide of arachidonic acid during prostaglandin formation in platelets [99–100]. The effect of 12-hydroperoxy arachidonic acid on platelet aggregation has not as yet been studied. Mickel & Horbar have reported vigorous platelet aggregation resulting from 15-hydroperoxy arachidonic acid.

Some observations that suggest this sequence of events are available. In perinatal telencephalic leukoencephalopathy, white matter damage in the neonatal infant is strongly correlated with the finding of postmortem gram-negative bacteremia [101]. Furthermore, intraperitoneal injection of endotoxin in the neonatal kitten results in significant white-matter

damage [102–103]. As in multiple sclerosis, so in infants dying with perinatal telencephalic leukoencephalopathy, a decrease in polyunsaturated fatty acids is found in white matter [104], an observation that can be explained by lipid peroxidation [47].

The marked adsorption of endotoxin onto platelets and the marked peroxidase activity in platelets suggests that platelets serve a scavenger function within the circulation [97]. With an overwhelming amount of endotoxin, there is development of endotoxic shock, possibly resulting from widespread peroxidative damage. On the other hand, with lesser amounts of endotoxin or other materials adsorbed onto platelets with resulting lipid peroxidation as a by-product of the platelet peroxidase, one can explain how affected platelets could pass through the lungs: peroxidation of platelet arachidonic acid would occur to a greater extent intraarterially, since a higher $pO_2$ occurs there. Furthermore, since myeloperoxidase and uterine peroxidase have been shown to be enhanced by estrogens [105–106], it is possible that platelet peroxidase is as well. The greater incidence of multiple sclerosis in females might be explained in this manner.

Enhanced platelet aggregation associated with adsorbed peroxidized arachidonic acid might be critical in the pathogenesis of multiple sclerosis. If platelets tend to adhere to each other, they are most likely to do so in those sites where there is greatest stasis, such as postcapillary venules. Aggregation of platelets there could result in the release of the peroxidized lipid. With adherence of platelets to the post capillary venular wall, the lipid peroxide might damage endothelial cells and adjacent oligodendroglial cells. Platelet thrombi as such need not occur. The observed perivenular distribution of demyelinating plaques in multiple sclerosis might be the result of lipid peroxidative attack on susceptible sulfhydryl groups of membrane proteins associated with endothelial and oligodendroglial cells.

The selective damage to white matter in multiple sclerosis might be the consequence of its susceptibility to peroxidative attack. Since myelin does not contain much polyunsaturated fatty acid [107], oligodendroglia might not require high intracellular concentrations of antioxidants to protect against the random endogenous formation of lipid peroxides. White matter might have little enzymatic capability of destroying lipid peroxides, since little lipid peroxide should be formed within myelin itself. Appreciable peroxidase activity has been found in periventricular glia and ependymodytes, but no mention is made of peroxidase activity in oligodendroglia [108–109]. Srebro, Cichocki, and Godula stated, "Although peroxidase activity in the brain is localized to only one or two types of glial cells, it is nonetheless constantly found at characteristic sites," namely, periventricularly [108]. With less peroxidase activity, oligodendroglia should not require as much protection against intracel-

lular peroxidation. With less protection against intracellular peroxidation, cells might become more vulnerable to damage from exposure to extracellular lipid peroxides, as is proposed here. The rarity of multiple sclerosis in childhood might be related to different enzymatic activities of oligodendroglia involved in the synthesis of myelin; that is, oligodendroglia might be less susceptible to exogenous peroxidative attack under these circumstances.

Enchanced platelet aggregation from peroxidized arachidonic acid could explain the abnormalities of small venules in multiple sclerosis suggested by the frequent occurrence of perivenous sheathing of the retina in the disease [18]. Roizin, Abell, and Winn examined blood flow through small conjunctival vessels under direct microscopy in multiple sclerosis patients and observed an increased tendency for cells to aggregate in the majority of their cases [110]. Abnormalities in nail-bed capillaries were demonstrated in multiple sclerosis patients by Gomirato [111]. Increased capillary fragility, which might be explained by damage to the capillary endothelium, was reported by Shulman et al. [112].

It has been shown that erythrocytes are also altered in this disease. Laszlo reported a highly significant increase in osmotic fragility of erythrocytes in multiple sclerosis [113], a finding confirmed by Caspary, Sewell, and Field [114]. Plum and Fog showed that the mean erythrocyte diameter was increased in multiple sclerosis [115]. This phenomenon can be explained by peroxidation of constituents of the erythrocyte membrane; conditions resulting in lipid peroxidation, such as exposure of erythrocytes to a pure oxygen atmosphere at high pressures either in vitro or in vivo, result in increased osmotic fragility and appreciable hemolysis [116–117].

As with the intraperitoneal injection of endotoxin in kittens [102–103], so the intraperitoneal injection of linoleate hydroperoxide in chicks, fed an antioxidant-deficient diet for 3–4 weeks containing sufficient linoleate to produce encephalomalacia, results in cerebral damage—including hemorrhage—and even death [118]. It is possible that this effect results from the transport of linoleate hydroperoxide to brain from the peritoneal cavity on platelet surfaces [86].

Should a peroxidative mechanism be implicated in multiple sclerosis, one might expect to see a decrease in the plasma lecithin–cholesterol acyltransferase reaction (plasma LCAT reaction), since it has been reported by us to be decreased by peroxidized lecithin in vitro and by conditions predisposing to lipid peroxidation in vivo [119–121]. No appreciable decrease in the plasma LCAT reaction is demonstrable in multiple sclerosis [122]. Also, we have unpublished results showing no appreciable effect of peroxidized arachidonic acid on the plasma LCAT reaction, although peroxidized lecithin produces a marked inhibition. The proposal that peroxidized arachidonic acid is implicated in multiple

sclerosis is not in conflict with the lack of reduction of the plasma LCAT reaction in multiple sclerosis. One might presume that peroxidized lecithin is not involved in multiple sclerosis. Stated simply, clinical manifestations associated with lipid peroxidation might depend upon the specific lipid peroxidized.

If peroxidized fatty acids were to attack sulfhydryl groups of proteins associated with endothelial or oligodendroglial cells, there might be denaturation of proteins and destruction of enzymes [51]. The denatured protein could serve as an antigen for the induction of an autoimmune process. An autoimmune response could result in the evolution of damage in the area of the demyelinating plaque. The infiltration of lymphocytes could also be accounted for. The elevation of gamma globulin in the cerebrospinal fluid in cases of chronic multiple sclerosis and the frequent absence of elevation during the initial attack might well be expected. In other words, there might be an inciting cause of multiple sclerosis related to a peroxidative mechanism and a secondary response related to autoimmunity. Exacerbations in multiple sclerosis might occur as a result of recurrence of a peroxidative process or the development of autoimmunity or both. The pathogenesis of chronic multiple sclerosis might then be different from that of the initial attack, in that autoimmunity could be predominant.

Another consequence of a peroxidative attack on white matter might be a decrease in the concentration of polyunsaturated fatty acids [47]. A significant decrease in polyunsaturated fatty acids has been observed by some in areas demyelinated and even in areas of no overt demyelination [25–28]. As one might expect in cases of chronic multiple sclerosis, no reduction in polyunsaturated fatty acids has been found [29–30].

The concept of lipid peroxidation and the effect of peroxidized arachidonic acid on platelets provides a means whereby one can account for diverse observations known about multiple sclerosis. The hypothesis incorporates much of the dietary, toxic, infectious, and autoimmune theories of the disease. The lipid peroxidative mechanisms outlined here could account for the parallel in epidemiology in Finland between multiple sclerosis and nutritional muscular dystrophy, another entity in which a peroxidative mechanism is presumably involved [123].

In summary, a diet rich in fat is likely to be rich in lipid peroxides. If there were altered metabolism in the intestinal tract, as with infection, it is possible that there would be increased absorption of these peroxides. Peroxides adsorbed onto platelets would increase their tendency to aggregate. Alternately, absorption of endotoxin or other material and its adsorption onto platelets might result in lipid peroxides by peroxidase activity. Aggregation of platelets might occur in the postcapillary venules of the brain, with release of the adsorbed peroxidized arachidonic acid,

which, in turn, crosses the "blood-brain barrier." White matter might be uniquely vulnerable to damage from peroxidized fatty acids. Enzymatic damage to endothelial cells and surrounding oligodendroglia could result from peroxidative attack on sulfhydryl groups and other nucleophiles. Denatured proteins, resulting from peroxidative attack, might serve as an antigen in the development of an autoimmune process, which, in turn, could result in further damage to white matter, possibly progressing to a chronic state of continuing damage to white matter.

## REFERENCES


1. J. W. Prineas. *In:* P. J. Vinken and G. W. Bruyn (eds.). Handbook of clinical neurology, vol. **9.** Amsterdam: North-Holland, 1970.
2. J. Cruveilhier. Anatomie pathologique du corps humain. Paris: Baillière, 1829–1842.
3. R. Carswell. Pathological anatomy: illustrations on the elementary forms of disease. London: Longman, Orme, Brown, Green, & Longman, 1838.
4. E.-F. Vulpian. Union Méd., **30:**459, 475, 541, 1866.
5. J.-M. Charcot. Prog. Med., Paris, **7:**97, 1879.
6. ———. *In:* G. Sigerson (trans.). Lectures on diseases of the nervous system delivered at the Salpetriere. London: New Sydenham Society, 1877.
7. ———. Gaz. Hop., Civils Milit., Paris, **41:**554, 1868.
8. P. Marie. Prog. Med., Paris, **12:**287, 305, 349, 365, 1884.
9. W. E. Bullock. Lancet, **2:**1185, 1913.
10. W. E. Gye. Brain, **44:**213, 1921.
11. H. Pette. World Neurol., **1:**491, 1960.
12. E. Pette and H. Pette. Dtsch. Z. Nervenheilkunde, **185:**295, 1963.
13. D. C. Gadjusek, C. J. Gibbs, Jr., and M. Alpers (eds.). Slow, latent, and temperature virus infections. Washington, D.C.: U.S. Department of Health, Education, and Welfare, 1965.
14. O. Marburg. Jahrb. Psychiatr. Neurol., **27:**213, 1906.
15. R. M. Brickner. Bull. Neurol. Inst. N.Y., **1:**105, 1931.
16. T. J. Putnam. N. Engl. J. Med., **209:**786, 1933.
17. ———. Arch. Neurol. Psychiatr., Chicago, **40:**806, 1938.
18. T. Fog. Acta Neurol. Scand., **41:**(suppl.):15, 1965.
19. R. L. Swank. A biochemical approach to multiple sclerosis. Springfield, Ill.: Thomas, 1961.
20. ———. Arch. Neurol. Psychiatr., Chicago, **73:**631, 1955.
21. R. H. S. Thompson. Proc. Roy. Soc. Med., **59:**269, 1966.
22. R. W. R. Baker, H. Sanders, R. H. S. Thompson, and K. J. Zilkha. J. Neurol. Neurosurg. Psychiatr., **28:**212, 1965.
23. R. W. R. Baker, R. H. S. Thompson, and K. J. Zilkha. J. Neurol. Neurosurg. Psychiatry, **27:**408, 1964.
24. ———. J. Neurol. Neurosurg. Psychiatr., **29:**95, 1966.
25. ———. Lancet, **1:**26, 1963.
26. B. Gerstl, M. C. Tavaststjerna, R. B. Hayman, L. F. Eng, and J. K. Smith. Ann. N.Y. Acad. Sci., **122:**405, 1965.

27. J. N. Cumings, R. C. Shortman, and T. Skrbic. J. Clin. Pathol., **18:**641, 1965.
28. G. Arnetoli, A. Pazzagli, and L. Amaducci. J. Neurochem., **16:**461, 1969.
29. I. Karlsson, C. Alling, and L. Svennerholm. Acta Neurol. Scand., **47:**403, 1971.
30. C. Alling, M.-T. Vanier, and L. Svennerholm. Brain Res., **35:**325, 1971.
31. I. R. Mackay and F. M. Burnet. Autoimmune diseases: pathogenesis, chemistry, and therapy. Springfield, Ill.: Thomas, 1964.
32. E. H. Farmer. Trans. Faraday Soc., **38:**340, 1942.
33. ———. Trans. Faraday Soc., **38:**356, 1942.
34. ———. Trans. Faraday Soc., **42:**228, 1946.
35. ———. Rubber Chem. Technol., **19:**267, 1946.
36. E. H. Farmer, G. F. Bloomfield, A. Sundralingam, and D. A. Sutton. Trans. Faraday Soc., **38:**349, 1942.
37. E. H. Farmer and A. Sundralingam. J. Chem. Soc., 121, 1942.
38. E. H. Farmer and D. A. Sutton. J. Chem. Soc., 139, 1942.
39. E. H. Farmer, H. P. Koch, and D. A. Sutton. J. Chem. Soc., 541, 1943.
40. W. Kern and L. Dulog. Makromolekulare Chem., **29:**199, 1949.
41. L. Bateman and J. L. Bolland. Proc. Int. Congr. Pure Appl. Chem., 11th Congr., **1:**325, 1947.
42. L. Bateman, J. L. Bolland, and G. Gee. Trans. Faraday Soc., **47:**274, 1951.
43. L. Bateman and G. Gee. Trans. Faraday Soc., **47:**155, 1951.
44. ———. Proc. Roy. Soc. A, **195:**391, 1948.
45. J. L. Bolland. Trans. Faraday Soc., **46:**358, 1950.
46. J. L. Bolland and G. Gee. Trans. Faraday Soc., **42:**236, 1946.
47. V. G. Parteshko, M. C. Pavlovskiai, and A. A. Lecuis. Dokl. Akad. Nauk SSSR, **205:**1248, 1972.
48. N. Uri. Autoxidation and antioxidants, vol. **1.** New York: Interscience, 1961.
49. S. E. Lewis and E. D. Wills. Biochem. Pharmacol., **11:**901, 1962.
50. I. D. Desai and A. L. Tappel. J. Lipid Res., **4:**204, 1963.
51. S. Capalna. Rev. Roum. Physiol., **8:**243, 1971.
52. R. T. Holman and S. I. Greenberg. Am. Oil Chem. Soc., **35:**707, 1958.
53. J. Glavind and N. Tryding. Acta Physiol. Scand., **49:**97, 1960.
54. V. Benes and B. A. J. Sedlacek. J. Hyg. Epidemiol. Microbiol. Immunol., Prague, **3:**96, 1959.
55. E. Degwitz and K. Lang. Fette, Seifen, Anstrichmittel, **64:**893, 1962.
56. D. C. Poskanzer, K. Schapira, and H. Miller. Lancet, **2:**917, 1963.
57. U. Leibowitz, E. Kahana, and M. Alter *In:* E. J. Field, T. M. Bell, and P. R. Carnegie (eds.). Multiple sclerosis: progress in research. Amsterdam: Elsevier, 1972.
58. G. Dean. *In:* E. J. Field, T. M. Bell, and P. R. Carnegie (eds.). Multiple sclerosis: progress in research. Amsterdam: Elsevier, 1972.
59. E. D. Acheson. *In:* E. J. Field, T. M. Bell, and P. R. Carnegie (eds.). Multiple sclerosis: progress in research. Amsterdam: Elsevier, 1972.
60. J. F. Kurtzke. *In:* E. J. Field, T. M. Bell, and P. R. Carnegie (eds.). Multiple sclerosis: progress in research. Amsterdam: Elsevier, 1972.
61. R. Eastman, J. Sheridan, and D. C. Poskanzer. N. Eng. J. Med., **289:**793, 1973.
62. M. Morrison, G. Bayse, and D. J. Danner. *In:* J. Schultz (ed.). Biochemistry of the phagocytic process. Amsterdam: North-Holland, 1970.

63. S. J. KLEBANOFF. *In:* J. SCHULTZ (ed.). Biochemistry of the phagocytic process. Amsterdam: North-Holland, 1970.
64. K. AGNER. Acta Physiol. Scand., **2** (suppl.):8, 1941.
65. J. SCHULTZ and K. KAMINKER. Arch. Biochem. Biophys., **96:**465, 1962.
66. J. SCHULTZ, R. CORLIN, F. ODDI, K. KAMINKER, and W. JONES. Arch. Biochem. Biophys., **111:**73, 1965.
67. D. F. BAINTON and M. G. FARQUHAR. J. Cell. Biol., **39:**286, 1968.
68. ———. J. Cell. Biol., **39:**299, 1968.
69. M. BAGGIOLIN, J. G. HIRSCH, and D. DEDUVE. J. Cell. Biol., **40:**529, 1969.
70. G. Y. N. IYER, D. M. F. ISLAM, and J. H. QUASTEL. Nature, **192:**535, 1961.
71. M. RECHCIGL and W. H. EVANS. Nature, **199:**1001, 1953.
72. J. ROBERTS and Z. CAMACHO. Nature, **216:**606, 1967.
73. B. PAUL and A. J. SBARRA. Biochim. Biophys. Acta, **156:**168, 1968.
74. M. ZATTI, F. ROSSI and P. PATRIARCA. Experientia, **24:**669, 1968.
75. J. BELIN, N. PETTET, A. D. SMITH, R. H. S. THOMPSON, and K. J. ZILKHA. J. Neurol. Neurosurg. Psychiatr., **34:**25, 1971.
76. N. TUNA, J. LOGOTHETIS, and R. KAMMERECK. Neurology, **13:**381, 1963.
77. H. SANDERS, R. H. S. THOMPSON, H. P. WRIGHT, and K. J. ZILKHA. J. Neurol. Neurosurg. Psychiatr., **31:**321, 1968.
78. S. GUL, A. D. SMITH, R. H. S. THOMPSON, H. P. WRIGHT, and K. J. ZILKHA. J. Neurol. Neurosurg. Psychiatr., **33:**506, 1970.
79. C. H. BOLTON, J. R. HAMPTON, and O. T. PHILLIPSON. Lancet, **1:**99, 1968.
80. M. NATHANSON and J. P. SAVITSKY. Bull. N.Y. Acad. Med., **28:**462, 1952.
81. T. FOG, I. KRISTENSEN, and H. F. HELWIG-LARSEN. Arch. Neurol., **73:**267, 1965.
82. E. A. CASPARY, J. PRINEAS, H. MILLER, and E. J. FIELD. Lancet, **2:**1108, 1965.
83. H. P. WRIGHT, R. H. S. THOMPSON, and K. J. ZILKHA. Lancet, **2:**1109, 1965.
84. J. H. D. MILLAR, J. D. MERRET, and A. M. DALBY. J. Neurol. Neurosurg. Psychiatr., **29:**187, 1966.
85. P. MILLAC. Dtsch. Z. Nervenheilkunde, **191:**74, 1967.
86. H. S. MICKEL and J. HORBAR. Lipids, **9:**68, 1974.
87. R. G. MASON, M. S. READ, S. R. SABA, and R. W. SHERMER. Thromb. Diath. Haemorrh., **27:**134, 1972.
88. M. OKUMA, M. STEINER, and M. BALDINI. Blood, **34:**523, 1969.
89. ———. J. Lab. Clin. Med., **75:**283, 1970.
90. O. BEHNKE. Anat. Rec., **158:**121, 1967.
91. E. ADELSON, J. J. RHEINGOLD, and W. H. CROSBY. Blood, **17:**767, 1961.
92. J. C. HOAD, A. A. SPECTOR, G. L. FRY, and B. C. BARNES. Blood, **40:**16, 1972.
93. J. BRETON-GORIUS and J. GUICHARD. Am. J. Pathol., **66:**277, 1972.
94. J. BRETON-GORIUS, M. T. DANIEL, G. FLANDRIN, and G. KINET DENOEL. Brit. J. Haematol., **25:**331, 1973.
95. M. OKUMA, M. STEINER, and M. G. BALDINI. J. Lab. Clin. Med., **77:**728, 1971.
96. M. NAGAYAMA, M. B. ZUCKER, and F. K. BELLER. Thromb. Diath. Haemorrh., **26:**467, 1971.
97. J. DAS, A. A. SCHWARTZ, and J. FOLKMAN. Surgery, **74:**235, 1973.
98. A. J. MARCUS, H. L. ULLMAN, and L. B. SAFIER. J. Lipid Res., **10:**108, 1969.
99. B. SAMUELSSON. 17th Int. Conf. Biochem. Lipids, Milan, September 9–11, 1974.
100. ———. 5th Int. Symp. Drugs Affecting Lipid Metab., Milan, September 9–12, 1974.
101. A. LEVITON and G. H. GILLES. J. Neurol. Sci., **18:**53, 1973.

102. F. H. Gilles, A. Leviton, and C. Kerr. Am. J. Pathol. (abstr.) (in press).
103. F. H. Gilles, D. Averill, and C. Kerr. J. Neuropath. Exp. Neurol. (abstr.) (in press).
104. H. S. Mickel, F. H. Gilles, J. Horbar, and P. Hill. J. Neuropath. Exp. Neurol., **33:**194, 1974.
105. A. A. Jacobs, R. J. Selvaraj, R. R. Strauss, B. B. Paul, G. W. Mitchell, Jr., and A. J. Sbarra. Am. J. Obstet. Gynecol., **117:**671, 1973.
106. R. H. Jellinck and C. R. Lyttle. Acta Endocrinol. (Kbh), **155** (suppl.): 122, 1971.
107. J. Clausen. *In:* A. Lajtha (ed.). Handbook of neurochemistry, vol. **1.** New York: Plenum, 1969.
108. Z. Srebro, T. Cichocki, and J. Godula. Acta Morphol. Acad. Sci. Hung., **19:**389, 1971.
109. Z. Srebro and T. Cichocki. Acta Histochem., **41:**108, 1971.
110. L. Roizin, R. C. Abell, and J. Winn. Neurology, **3:**250, 1953.
111. G. Gomirato. Riv. Patol. Nerv. Ment., **53:**148, 1939.
112. H. H. Shulman, L. Alexander, O. F. Ehrentheil, and R. Gross. J. Neuropathol. Exp. Neurol., **9:**420, 1950.
113. S. Laszlo. Acta Neurol. Psychiatr. Belg., **64:**529, 1964.
114. E. A. Caspary, F. Sewell, and E. J. Field. Br. Med. J. **2:**610, 1967.
115. C. M. Plum and T. Fog. Acta Psychol. Scand., **34** (suppl.): 128, 1959.
116. C. E. Mengel and H. E. Kann. J. Clin. Invest., **45:**1150, 1966.
117. C. E. Tsen and H. B. Collier. Can. J. Biochem., **38:**981, 1966.
118. T. Nishida, H. Tsuchiyama, M. Inoue, and F. A. Kummerow. Proc. Soc. Exp. Biol. Med., **105:**308, 1960.
119. H. S. Mickel, E. L. Foulds, and D. A. Clark. Lipids, **7:**121, 1972.
120. H. S. Mickel, E. L. Foulds, D. A. Clark, and E. C. Larkin. Lipids, **6:**740, 1971.
121. H. S. Mickel, K. C. Hayes, and P. L. Hill. J. Am. Oil Chem. Soc., **50:**88A, 1973.
122. G. E. Demedig, L. Amaducci, P. Borri, A. Gaiti, and G. Procellati, Lancet, **2:**1233, 1972.
123. J. Palo, J. Wikstrom, and E. Kivalo. Lancet, **2:**848, 1973.

# REDUCTION OF CHOLESTEROL ESTERIFICATION IN PATIENTS WITH CYSTIC FIBROSIS AND REDUCED PLASMA VITAMIN E CONCENTRATIONS

Hubert S. Mickel, M.D.,[1] Penelope L. Hill, M.D.,[2]
K.C. Hayes, D.V.M., Ph.D.,[3] and Harry Swachman, M.D.[4]

1,2 Department of Neurology
Children's Hospital Medical Center
Boston, Massachusetts 02115

3 Department of Nutrition
Harvard School of Public Health
Boston, Massachusetts 02115

4 Department of Medicine
Children's Hospital Medical Center
Boston, Massachusetts 02115

Supported in part by grants-in-aid from the Office of Naval Research, Department of the Navy, N00014-75-C-0719, NR202-017; the United Cerebral Palsy Research and Educational Foundation, R224-69; the National Institutes of Health, NS-HD-09704 and HD-03773 (Dr. Mickel) and HL-10098, HL-15797 and K04-HL-70285 (Dr. Hayes); the Fund for Research and Teaching, Department of Nutrition, Harvard School of Public Health; the Ira Sue Perlmutter Research Fund; and the Hearst Fund.

Correspondent: Hubert S. Mickel, M.D.
Department of Neurology
Children's Hospital Medical Center
Boston, Massachusetts 02115

(617)-734-6000 (x2891)

SUMMARY

The present study compares cholesterol esterification, vitamin E levels, carotene levels, phospholipid levels, and total cholesterol levels in sera of patients with cystic fibrosis and of unaffected individuals.

There is a reduction in the extent of esterification of cholesterol by the plasma lecithin-cholesterol acyltransferase (LCAT) reaction in patients with cystic fibrosis, compared with unaffected controls. An unequivocal reduction in vitamin E concentration as well as in carotene concentration was also associated with the cystic fibrosis group. Both cholesterol and phospholipid concentrations were reduced in the cystic fibrosis group, although the extent of reduction was less marked than the vitamin E and carotene levels.

The reduced LCAT esterification could result from poor absorption of tocopherol from the intestine and subsequent peroxidative damage to the sulfhydryl groups associated with the enzyme as well as from reduced substrate concentrations resulting from poor absorption from the intestine.

## SPECULATION

The observed reduced lecithin-cholesterol acyltransferase esterification in cystic fibrosis could be the result of poor absorption of vitamin E from the intestine and subsequent peroxidative damage to the sulfhydryl groups associated with the enzyme as well as be the result of reduced substrate concentrations, also resulting from their poor absorption from the intestine.

## INTRODUCTION

The reduction in plasma $\alpha$-tocopherol concentrations, which has been reported in cystic fibrosis (8), has been attributed to poor absorption of fat-soluble materials from the intestine (10). Reduced cholesterol esterification by the plasma lecithin-cholesterol acyltransferase reaction is associated with vitamin E deficiency in the monkey (6,7) and in the rat (11). The present study compares cholesterol esterification, vitamin E levels, carotene levels, phospholipid levels, and total cholesterol levels in sera of patients with cystic fibrosis and of unaffected individuals.

## MATERIALS AND METHODS

Sera from eight patients with cystic fibrosis and from four unaffected controls were assayed for cholesterol esterification by the lecithin-cholesterol acyltransferase (LCAT) reaction. The increase in concentration of cholesterol esters in serum was determined after a 24 hour incubation at 37 C. Lipids were extracted from the serum samples before and after incubation with chloroform : methanol (2:1, v/v). Chromatographic separation of cholesterol and cholesterol esters was accomplished with 1 gram silica gel columns, using the method of Hirsch and Ahrens (3). Cholesterol concentration was determined by the method of Rosenthal, Pfluke, and Busacaglia (9). Vitamin E and carotene levels were determined by the fluorometric method of Hansen and Warwick (2). Phospholipid levels were determined by the method of Bartlett (1).

## RESULTS

There is a reduction in the extent of esterification of cholesterol by the plasma lecithin-cholesterol acyltransferase (LCAT) reaction in patients with cystic fibrosis, compared with unaffected controls. An unequivocal reduction in vitamin E concentration as well as in carotene concentration was also associated with the cystic fibrosis group. Both cholesterol and phospholipid concentrations were reduced in the cystic fibrosis group, although the extent of reduction was less marked than were the vitamin E and carotene levels.

Refer to the Table.

## DISCUSSION

Since decreased cholesterol esterification has been demonstrated in vitamin E deficiency (6,7,11), the reduction in cholesterol esterification in sera from patients with cystic fibrosis may also be related to the observed decreased levels of vitamin E. The reduction in cholesterol esterification might be related to a reduction in the activity of the enzyme itself.

Plasma lecithin-cholesterol acyltransferase esterification is sensitive to conditions predisposing to lipid peroxidation. LCAT esterification is decreased on *in vivo* exposure to a pure oxygen atmosphere in humans (4), and the sulfhydryl groups of the enzyme are susceptible to peroxidative attack *in vitro* (5). If vitamin E protects against *in vivo* lipid peroxidation, then a reduction in vitamin E levels would predispose to a reduction in activity of those enzymes which are susceptible to such damage.

Reduction in phospholipid levels and cholesterol levels in cystic fibrosis offers an alternative explanation for the observed reduction in cholesterol esterification in sera. A reduction in substrate concentrations will result in a reduction in the amount of cholesterol esterified.

The role of lipid peroxidation in vitamin E deficiency has not been fully established, so that additional information is required before one can categorically attribute observed reductions in cholesterol esterification in cystic fibrosis to observed reductions in vitamin E levels, especially in the presence of reduced substrate levels.

COMPARISON OF CHOLESTEROL ESTERIFICATION, VITAMIN E LEVELS, AND SERUM LIPIDS IN PATIENTS WITH CYSTIC FIBROSIS AND IN UNAFFECTED INDIVIDUALS

CYSTIC FIBROSIS

| Vitamin E ug/100 ml | Carotene ug/100 ml | Phospholipids mg/100 ml | Total Cholesterol mg/100 ml | Cholesterol Esterified mg/100 ml |
|---|---|---|---|---|
| 300 | 210 | 84 | 129 | 21.7 |
| 100 | 50 | 141 | 96 | 21.3 |
| 100 | 100 | 182 | 105 | 41.8 |
| 300 | 80 | 137 | 127 | 45.7 |
| 200 | 120 | 173 | 211 | 36.3 |
| 100 | 70 | 215 | 121 | 15.9 |
| 100 | 90 | 131 | 130 | 29.3 |
| 200 | 110 | 164 | 183 | 30.9 |
| 175±89[a] | 104±48 | 153±39 | 138±39 | 30.4±10.5 |

UNAFFECTED INDIVIDUALS

| Vitamin E ug/100 ml | Carotene ug/100 ml | Phospholipids mg/100 ml | Total Cholesterol mg/100 ml | Cholesterol Esterified mg/100 ml |
|---|---|---|---|---|
| 400 | 300 | 143 | 137 | 37.3 |
| 550 | 220 | 178 | 310 | 77.7 |
| 900 | 430 | 203 | 223 | 55.3 |
| 250 | 150 | 217 | 236 | 84.6 |
| 525±278 | 275±120 | 185±32 | 227±71 | 63.7±21.6 |
| <0.005[b] | <0.005 | <0.10 | <0.01 | <0.005 |

[a] Mean±standard deviation

[b] P value determined by student's "t" test

REFERENCES

1. Bartlett, G.R., Phosphorus assay in column chromatography. J. Biol. Chem., 234: 466 (1959).
2. Hansen, L.G., and Warwick, W.J., A fluorometric micro method for serum vitamins A and E. Amer. J. Clin. Path., 51: 538 (1969).
3. Hirsch, J., and Ahrens, E.H., The separation of complex lipid mixtures by use of silicic acid chromatography. J. Biol. Chem., 233: 311 (1958).
4. Mickel, H.S., Foulds, E.L., Clark, D.A., and Larkin, E.C., Effects of pure oxygen atmosphere *in vivo* on plasma lecithin-cholesterol acyltransferase reaction. Lipids, 6: 740 (1971).
5. Mickel, H.S., Foulds, E.L., and Clark, D.A., Inhibition of the plasma lecithin-cholesterol acyltransferase reaction by hydrogen peroxide and peroxidized lecithin. Lipids, 7: 121 (1972).
6. Mickel, H.S., Hayes, K.C., and Hill, P.L., Depression of the lecithin-cholesterol acyltransferase reaction in vitamin E-deficient monkeys. J. Amer. Oil Chem. Soc., 50: 88A (1973).
7. Mickel, H.S., Hill, P.L., and Hayes, K.C., Depression of lecithin-cholesterol acyltransferase esterification in vitamin E-deficient monkeys. Amer. J. Clin. Nutr., 28: 994 (1975).
8. Muller, D.P.R., and Harries, J.T., Vitamin E studies in children with malabsorption. Biochem. J., 122: 28 P (1969).
9. Rosenthal, H.L., Pfluke, M.L., and Busacaglia, S., A stable iron reagent for determination of cholesterol. J. Lab. Clin. Med., 50: 318 (1957).
10. Schnitzer, B., and Loesel, L.S., Brown bowel. Amer. J. Clin. Pathol. 50: 433 (1968).
11. Takatori,T., and Privett, O.S., Studies of serum lecithin-cholesterol acyltransferase activity in rat: Effect of vitamin E deficiency, oxidized dietary fat, or intravenous administration of ozonides or hydroperoxides. Lipids, 9: 1018 (1974).

# DECREASE IN POLYUNSATURATED FATTY ACIDS IN WHITE MATTER IN PERINATAL TELENCEPHALIC LEUKOENCEPHALOPATHY

Hubert S. Mickel, M.D., Penelope L. Hill, M.D.
Jeffrey Horbar, and Floyd H. Gilles, M.D.

From the Departments of Neurology and Pathology (Neuropathology), The Children's Hospital Medical Center; and the Department of Neurology-Neuropathology, Harvard Medical School, Boston, Massachusetts 02115.

Page Proof Recipient: Hubert S. Mickel, M.D.
Department of Pathology (Neuropathology)
The Children's Hospital Medical Center
Boston, Massachusetts 02115
U.S.A.

ACKNOWLEDGEMENTS

This study was supported in part by funds provided by the United Cerebral Palsy Research and Educational Foundation (R-224-74), The Children's Hospital Medical Center Mental Retardation and Human Development Research Program (5 P30 HD0 6276) and 5 P01 NS09704, NINCDS. Funds were also provided by the Office of Naval Research, Department of the Navy, N00014-75-C-0719, NR202-017.

## Abstract

Decreased percentage of linoleic acid and arachidonic acid was found in the total fatty acids of the white matter lipids in perinatal telencephalic leukoencephalopathy, which is a common abnormality in white matter in neonatal humans. A pathogenetic model is proposed for these observations.

INTRODUCTION

Damage in developing white matter in infants free of diseases considered neurotoxic was described as consisting of hypertrophic astrocytes, amphophilic globules, necrotic foci, and acutely damaged glia in white matter (Gilles and Murphy, 1969). Because of clustering of histologic features, the morphologic definition was subsequently narrowed to the cluster of hypertrophic astrocytes and amphophilic globules (Leviton and Gilles, 1971). The entity is called perinatal telencephalic leukoencephalopathy. Hypertrophic astrocytes are cells with pale vesicular nuclei and eosinophilic irregular hyaline cytoplasm. Such cells do not occur in normal human myelinating white matter (Mickel and Gilles, 1970). Amphophilic globules are PAS positive, protein-rich, mineralized deposits located in or near capillaries or venules.

Terminal gram-negative bacteremia is associated with perinatal telencephalic leukoencephalopathy by secular trends and high risk ratio (Leviton and Gilles, 1973). Further, exposure to endotoxin in the newborn kitten induces a leukoencephalopathy which is characterized by cellular astrogliosis with hypertrophic astrocytes, focal areas of cystic necrosis, and deposits of globular debri, either mineralized or eosinophilic (Gilles et al., 1977).

Endotoxin has been postulated as involved in the pathogenesis of damage to myelinated white matter in multiple sclerosis, by inciting platelets to produce peroxidized arachidonic acid, which, in turn, results in peroxidative damage to endothelium and to perivenular oligodendroglia (Mickel,

1975). In this model, polyunsaturated fatty acids would be consumed by a peroxidative process (Parteshko et al, 1972) and would account for the decrease in polyunsaturated fatty acids in uninvolved white matter in multiple sclerosis (Baker, 1963; Gerstl et al., 1965; Cumings et al., 1965; Arnetoli et al., 1969). A decrease in polyunsaturated fatty acids in white matter of infants dying with perinatal telencephalic leukoencephalopathy would be compatible with an analogous lipid peroxidative model.

MATERIALS AND METHODS

Three cases of perinatal telencephalic leukoencephalopathy with total ages (gestational age plus survival age) of 34, 45, and 46 weeks were compared with four controls with total ages of 33, 35, 45, and 48 weeks. The mean age for the cases with perinatal telencephalic leukoencephalopathy was 41.3 weeks and for the control group was 40.3 weeks.

The frontal pole of each brain was removed at the time of autopsy and frozen at -85° C without fixation. When studied, the brain was allowed to warm, but while still frozen, white matter was dissected free of grey matter, weighed, homogenized and extracted according to the method of Folch-Pi and Lees (1951). Methyl esters of the fatty acids were prepared from the total lipids using boron trifluoride and methanol. Gas chromatographic separation was carried out using columns of 10% diethylene glycol succinate on Anakron SD, 80-100 mesh, with helium as carrier gas, at a temperature of 195°C. The percentages were calculated from the relative areas of the peaks using an Infotronics integrator.

RESULTS

Perinatal telencephalic leukoencephalopathy brains have decreased levels of linoleic acid (18:2) and arachidonic acid (20:4) in the white matter, compared with unaffected controls of comparable age range. Linolenic acid (18:3) was not detected in any of the white matter samples.

| CONTROL | LINOLEIC ACID | ARACHIDONIC ACID |
|---|---|---|
| TOTAL AGE (Weeks) | | |
| a) 33 | 0.42% | 7.36% |
| b) 35 | 0.58% | 8.39% |
| c) 45 | 1.27% | 9.82% |
| d) 48 | 1.52% | 12.43% |
| MEAN ± S.D. | 0.95% ± 0.53% | 9.50% ± 2.20% |
| PERINATAL TELENCEPHALIC LEUKOENCEPHALOPATHY | | |
| TOTAL AGE (Weeks) | | |
| e) 34 | 0.00% | 9.67% |
| f) 45 | 0.48% | 8.16% |
| g) 46 | 0.00% | 0.80% |
| MEAN ± S.D. | 0.16% ± 0.27% | 6.21% ± 4.75% |

Using the student's t test, the difference in percentage between the two groups is significant. For linoleic acid $P < 0.005$ and for arachidonic acid, $P < 0.01$.

DISCUSSION

Although decreased levels of polyunsaturated fatty acids have been demonstrated in white matter without focal necrosis, these results must be interpreted with caution in view of the relatively small number of cases. However, the finding of decreased levels of polyunsaturated fatty acids in the white matter of brains with perinatal telencephalic leukoencephalopathy supports the hypothesis that a peroxidative mechanism, induced by endotoxin, is implicated.

Exposure to endotoxin produces white matter damage in the newborn kitten, the newborn rat, as well as the newborn monkey. Morphological findings in the neonatal monkey consist acutely, of small diapedetic hemorrhages surrounding many of the small vessels in the white matter, whereas other small vessels are surrounded by pools of eosinophilic, presumably proteinaceous material or small, eosinophilic globules (Gilles et al., 1977). After 1-2 weeks following exposure to the endotoxin, the lesions were still pericapillary and perivenular in location.

A perivenular location occurs with the small demyelinating lesions of multiple sclerosis in adult human white matter (Putnam, 1933; Fog, 1950; Fog, 1964; Fog, 1965). Decreased levels of linoleic acid in the plasma (Thompson, 1966; Baker et al., 1965; Baker et al., 1964; Baker, 1966; Tichy and Vymazal, 1973) and in the white matter (Baker, 1963; Gerstl et al., 1965; Cumings et al., 1965; Arnetoli et al., 1969) in patients with acute multiple sclerosis suggested that a peroxidative mechanism might be involved in the pathogenesis of multiple sclerosis

(Mickel, 1975). The absence of these decreases in polyunsaturated fatty acids in chronic multiple sclerosis (Alling et al., 1971; Karlsson et al., 1971) is not contradictory, since a peroxidative mechanism is proposed only as an inciting event.

Endotoxin has a marked affinity for adsorption onto platelet surfaces (Nagayama et al., 1971; Das et al., 1973) where it, like a number of foreign materials, might result in increasing amounts of lipid peroxides (Okuma et al., 1969; Okuma et al., 1969; Okuma et al., 1971). Arachidonic acid is most likely to be peroxidized in the platelet, since it is present in the highest concentration of any polyunsaturated fatty acid in platelet phospholipids (Marcus et al., 1969). Arachidonic acid is the only fatty acid, which, when peroxidized, induces platelet aggregation (Mickel and Horbar, 1974).

Endotoxin proposed as a possible agent to induce the platelet to form peroxidized arachidonic acid, which in turn, can be adsorbed onto human platelet <u>in vitro</u> without destruction of the labile peroxyl group (Mickel and Horbar, 1974). Peroxidized lipid might then be carried on the surface of platelets to the brain, where, in capillaries and venules, aggregation of platelets would result in release of the peroxidized lipid and damage the surrounding endothelial and oligodendroglial cells.

Supporting the notion that lipid peroxides are involved in the white matter damage induced by endotoxin is the observation of Nishida et al., (1960), in which the intravenous injection

into chicks of linoleate hydroperoxide resulted in ataxia and white matter damage. It is possible that the peroxidized lipid was transported to the brain on the surfaces of platelets.

Our intention in describing these decreases in the relative amounts of polyunsaturated fatty acid in brains of infants with perinatal telencephalic leukoencephalopathy is to suggest further a possible mechanism of damage to white matter to this disease of unmyelinated white matter.

REFERENCES

Alling, C., M.-T. Vanier, and L. Svennerholm. (1971): Lipid alterations in apparently normal white matter in multiple sclerosis. Brain Res. 35, 325-336.

Arnetoli, G., A. Pazzagli, and L. Amaducci. (1969): Fatty acid and aldehyde changes in choline- and ethanolamine-containing phospholipids in white matter of multiple sclerosis brains. J. Neurochem. 16, 461-463.

Baker, R.W.R., R.H.S. Thompson, and K.J. Zilkha. (1963): Fatty acid composition of brain lecithins in multiple sclerosis. Lancet 1, 26-27.

Baker, R.W.R., R.H.S. Thompson, and K.J. Zilkha. (1964): Serum fatty acids in multiple sclerosis. J. Neurol. Neurosurg. & Psychiat. 27, 408-414.

Baker, R.W.R., H. Sanders, R.H.S. Thompson, and K.J. Zilkha. (1965): Serum cholesterol linoleate levels in multiple sclerosis. J. Neurol. Neurosurg. & Psychiat. 28, 212-217.

Baker, R.W.R., R.H.S. Thompson, and K.J. Zilkha. (1966): Changes in the amounts of linoleic acid in the serum of patients with Multiple sclerosis. J. Neurol. Neurosurg. & Psychiat. 29, 95-98.

Cumings, J.N., R.C. Shortman, and T. Skrbic. (1969): Lipid studies in the blood and brain in multiple sclerosis and motor neuron disease. J. Clin. Pathol. 18, 641-644.

Das, J., A.A. Schwartz, and J. Folkman. (1973): Clearance of endotoxin by platelets: Role in increasing the accuracy

sclerosis and other neurological diseases. Acta Neurol. Scand. 47, 403-412.

Leviton, A., and F.H. Gilles. (1971): Clustering of the morphological components of perinatal telencephalic leukoencephalopathy. J. Neurol. Neurosurg. & Psychiat. 34, 642-645.

Leviton, A., and F.H. Gilles. (1973): An epidemiological study of perinatal telencephalic leukoencephalopathy in an autopsy population. J. Neurol. Sci. 18, 53-66.

Marcus, A.J., H.L. Ullman, and L.B. Safier. (1969): Lipid composition of subcellular particles of human blood platelets. J. Lipid Res. 10, 108-114.

Mickel, H.S., and F.H. Gilles. (1970): Changes in glial cells during human telencephalic myelinogenesis. Brain 93, 337-346.

Mickel, H.S., and J. Horbar. (1974): The effect of peroxidized arachidonic acid upon human platelet aggregation. Lipids 9, 68-71.

Mickel, H.S. (1975): Multiple sclerosis: a new hypothesis. Perspect. Biol. & Med. 18, 363-374.

Nagayama, N., M.B. Zucker, and F.K. Beller. (1971): Effects of a variety of endotoxins on human and rabbit platelet function. Thromb. Diath. Haemorrh. 26, 467-473.

Nishida, T., H. Tsuchiyama, M. Inoue, and F.A. Kummerow. (1960): Effect of intravenous injection of oxidized methyl esters of unsaturated fatty acids on chick encephalomalacia. Proc. Soc. Exp. Biol. & Med. 105, 308-312.

Okuma, M., M. Steiner, and M. Baldini. (1969): Lipid peroxidation in blood platelets. Blood 34, 523-524.

Okuma, M., M. Steiner, and M. Baldini. (1969): Lipid peroxidation in aging platelets. Blood 34, 712-716.

Okuma, M., M. Steiner, and M. Baldini. (1971): Studies on lipid peroxides in platelets II. Effect of aggregating agents and platelet antibody. J. Lab. Clin. Med. 77, 728-742.

Parteshko, V.G., M.C. Pavlovskiai, and A.A. Lesiuis. (1972): Decrease of polyunsaturated fatty acid level in the liver of animals as a consequence of the free-radical process intensification in biolipids under the effect of exogenous lipoperoxides. Dokl. Akad. Nauk. SSSR. 205, 1248-1250.

Putnam, T.J. (1933): The pathogenesis of multiple sclerosis: a possible vascular factor. N. Engl. J. Med. 209, 786-790.

Thompson, R.H.S. (1966): A biochemical approach to the problem of multiple sclerosis. Proc. Roy. Soc. Med. 59, 269-276.

Tichy, J., and J. Vymazal. (1973): Changes of some serum fatty acids and lipids in relation to the clinical course of multiple sclerosis. Acta Neurol. Scand. 49, 345-354.

# Depression of lecithin-cholesterol acyltransferase esterification in vitamin E-deficient monkeys[1, 2]

*Hubert S. Mickel,[3] M.D., Penelope L. Hill,[4] B.A., and K. C. Hayes,[5] D.V.M., Ph.D.*

ABSTRACT Vitamin E deficiency in two species of monkeys (capuchins and cynamolgus) reduced the in vitro cholesterol esterification by plasma lecithin–cholesterol acyltransferase. The reduction was greatest in the most deficient species and in animals fed a diet rich in polyunsaturated fat (safflower oil) stripped of vitamin E. The best correlate of total esterification was the plasma concentration of free cholesterol which reflected the degree of hyperlipidemia, found to be greatest in capuchins fed coconut oil. A logical explanation for the decreased LCAT activity in vitamin E deficiency would be peroxidative damage of substrate (the PUFA of lecithin) or of sulfhydryl sites on lecithin–cholesterol acyltransferase itself. However, neither case was fully supported by the data suggesting that additional information concerning the nature of the reaction and the role of vitamin E is required. *J. Clin. Nutr.* 28: 994–999, 1975.

Vitamin E has been considered a physiological lipid antioxidant which protects against continual, random, free radical-catalyzed lipid peroxidation (1). Presumably due to the increased incorporation of polyunsatured fatty acids (PUFA) into tissue lipids during consumption of a high PUFA diet, a direct relationship between dietary unsaturated fat and the occurrence of vitamin E-deficiency disease has been established (2). It has also been proposed that vitamin E may function as a catalytic agent in the intermediary metabolism of mitochondria, where it has a direct and immediate effect on terminal respiration. Unrelated to genetic transcription, this effect has been described as the antioxidant sparing of sulfhydryl sites or other highly sensitive loci on enzymes indispensable to respiration (3). In vitamin E deficiency there is a decrease in the activity of the enzymes not specific to energy metabolism, suggesting that tocopherol may protect enzymes susceptible to peroxidative attack. In vitro studies of enzyme inactivation by lipid peroxidation have shown sulfhydryl enzymes are most susceptible to this form of inactivation (4, 5). If vitamin E protects against in vivo lipid peroxidation, and if lipid peroxidation inactivates sulfhydryl-containing enzymes, one might expect a decrease in plasma lecithin–cholesterol acyltransferase (LCAT) activity in vitamin E deficiency. Plasma LCAT depends on sulfhydryl sites since its activity is lost on exposure to *p*-chloromercuribenzoate (6). Furthermore, the LCAT reaction was markedly inhibited by hydrogen peroxide or peroxidized lecithin (7, 8) and activity was depressed in vivo in men exposed to a pure oxygen atmosphere, a condition predisposing to lipid peroxidation (9). A decrease in the plasma LCAT reaction has been observed in humans suffering from abetalipoproteinemia (10), a disease process associated with low circulating vitamin E concentrations and hypolipidemia in general (11, 12).

This study was undertaken to determine whether there was any decrease in the esterification of cholesterol in plasma by the LCAT

---

[1] From the Department of Neurology, Children's Hospital Medical Center and the Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts

[2] Supported in part by grants-in-aid from the Office of Naval Research Department of the Navy, NR-908-108; the United Cerebral Palsy Research and Educational Foundation, R224-69; the National Institutes of Health, NS-HD-09704 and HD-03773 (Dr. Mickel) and HL-10098, HL-15797 and KO4-HL-70285 (Dr. Hayes); and the Fund for Research and Teaching, Department of Nutrition, Harvard School of Public Health. This paper was presented in part at the 64th Annual Spring Meeting of the American Oil Chemists' Society, April 29–May 2, New Orleans, Louisiana.

[3] Assistant Professor Neurology, Children's Hospital Medical Center. [4] Research Fellow, Children's Hospital Medical Center. [5] Assistant Professor of Nutrition, Harvard School of Public Health.

reaction in monkeys made deficient in vitamin E and fed diets containing saturated or polyunsaturated fats. Reduction in the plasma LCAT reaction in vitamin E deficiency would support the concept that one of the metabolic effects of the vitamin is protection of the activity of the sulfhydryl-containing enzymes by prevention of lipid peroxidation.

## Materials and methods

A total of 26 Old and New World infant monkeys were selected from monkeys born in the primate breeding colony of the Department of Nutrition at the Harvard School of Public Health. The 13 cynamolgus (*Macaca fascicularis*) and 13 capuchins (*Cebus albifrons* and *apella*) were removed from their dams at birth and raised in the infant nursery (13). For the first 12 months of life all the monkeys were used in studies related to protein requirements for growth. Thereafter, these animals were randomized as to sex and species and assigned to one of four dietary treatment groups varied as to dietary fat (saturated coconut oil or polyunsaturated, stripped safflower oil) and vitamin E content (no vitamin E supplement or added vitamin E). The composition of the diet has been previously reported (14). The diets were prepared every 10–14 days in the form of agar cakes and were fed ad libitum allowing 200 kcal/kg body weight per day for each animal. For the first 50 weeks of the study, 0.2% cholesterol was added to the diet as an atherogenic stress. It was removed at the start of the 51st week. The monkeys were housed in individual cages in an air-conditioned room maintained at 22.2 ± 1.1 C. Body weights were recorded biweekly.

A 16-hour fasting blood sample was drawn from the femoral vein into an EDTA wetted syringe routinely at 16-week intervals to monitor hematologic parameters, and periodic vitamin E concentrations (14). After 63 weeks, plasma was similarly collected for assay of the plasma LCAT enzyme.

To assay the extent of LCAT esterification (as opposed to initial rate) (15), 1 ml of a solution of 4-$^{14}$C-cholesterol in hexane, containing 0.08 $\mu$Ci (2.6 × $10^{-3}$ $\mu$moles or 1.01 $\mu$g) cholesterol, was added to each incubation vial and evaporated to dryness under a stream of nitrogen. An aliquot of 0.8 ml of plasma was added to each vial which was promptly capped and incubated for approximately 24 hours at 37 C with continous shaking. After incubation, the lipids were extracted with chloroform:methanol (2:1 v/v) in 25-ml volumetric flasks. A zero time control of 0.5 ml of plasma was extracted similarly prior to incubation of each sample. An aliquot of 20 ml from each incubated and nonincubated plasma extract was evaporated to dryness under reduced pressure and temperature, and the residual lipids were redissolved in 3 ml purified petroleum ether. The extracted lipids were added to 1 g silica gel columns in 1 ml petroleum ether using the method of Hirsch and Ahrens to separate the free cholesterol and cholesteryl ester fractions (16). Radioactivity in each column fraction was measured by evaporating an aliquot of the column effluent in a scintillation vial and counting with Permafluor in toluene in a Packard Tri-carb scintillation counter. The cholesterol content of unesterified and esterified cholesterol fractions, in both incubated and nonincubated samples, was determined using the method of Rosenthal et al. (17).

The extent of esterification by LCAT was calculated from the cholesteryl esters formed as measured by two methods, the first being the degree of esterification of radioactive cholesterol substrate and the second a colorimetric determination of the net increase in cholesteryl ester concentration during incubation. The values obtained by the colorimetric method were higher since the radioisotope method measures only exogenous labeled cholesterol that must first be incorporated into lipoprotein before it can be esterified (8, 15). Since analysis of variance indicated that the significance of the data obtained by both methods was comparable, only the data from the radioisotope method are included.

Methyl esters of fatty acids of the plasma cholesterol esters were prepared by saponifying the plasma with 0.5 N NaOH, then methylating the free fatty acids using 14% $BF_3$ in methanol (v/v) (Supelco, Bellefonte, Penn.). Analysis of the methyl esters was performed on a Nuclear-Chicago 5,000 gas chromatograph on a 6-ft glass column (3 mm in diameter) packed with 10% DEGS (diethyleneglycol succinate) on Anakrom SD 100/110 (Analabs, North Haven, Conn.). Integration of peak areas was determined by interfacing the gas chromatograph with an Infotronics digital integrator (model CRS-208).

Statistical analysis was accomplished using a standard computerized Datatext program for analysis of variance of dietary fat, vitamin E status, and species. A similar program was used to derive correlation coefficients between LCAT activity and various other parameters measured.

## Results

During the course of the 63-week period prior to sampling for plasma LCAT activity, one animal died from anesthesia and during the 34th week two sudden deaths occurred in monkeys fed the safflower oil diet deficient in vitamin E. At the time of bleeding, there were 5 animals in the group fed the safflower oil diet supplemented with vitamin E (3 cynamolgus and 2 capuchins) and 6 animals in the group fed safflower oil without tocopherol (2 cynamolgus and 4 capuchins). In the groups fed coconut oil diets, there were 6 monkeys receiving a vitamin E supplement (3 cynamolgus and 3 capuchins) and 6 not receiving vitamin E (4 cynamolgus and 2 capuchins). No specific signs of vitamin E deficiency were apparent nor was an anemia present at this time, although vitamin E-deficient monkeys tended to have lower hematocrits and deficient monkeys of both species eventually developed a marked hemolytic anemia. The plasma vitamin E concentrations were low in

the unsupplemented monkeys several weeks prior to this assay, whereas supplemented animals receiving 100 mg/kg diet at the time of the assay were found to be adequate at subsequent intervals. Cynamolgus monkeys had lower vitamin E concentrations than capuchins. The data on the anemia and plasma vitamin E concentrations have been presented elsewhere (14).

A significant reduction ($P < 0.001$) in the amount of cholesterol esterified by plasma LCAT was observed in the vitamin E-deficient monkeys, both the cynamolgus and capuchins. This reduction was most pronounced in those animals fed the diet containing safflower oil. Also, the capuchins fed coconut oil had more than twice the esterifying activity of those capuchins fed safflower oil. This coconut oil effect was much less noticeable among the cynamolgus (Table 1). Furthermore, those capuchins fed coconut oil plus vitamin E demonstrated twice the esterifying activity of comparable cynamolgus, whereas comparison of esterification between the two species for the monkeys fed safflower oil showed no differences. On an overall basis the LCAT activity was highly correlated with the concentration of plasma free cholesterol ($r = 0.83$) and total cholesterol ($r = 0.68$) and less significantly with cholesteryl esters ($r = 0.61$). The cholesterol values were, in turn, a reflection of the dietary fat consumed. On considering the LCAT activity in terms of the percent of free cholesterol esterified in 24 hours, it became apparent that there was no dietary effect among the cynamolgus (all esterified about 30%), whereas the capuchins fed coconut oil plus vitamin E demonstrated an enhanced percent esterification (50%)—roughly double that of any other group of monkeys.

Vitamin E deficiency tended to lower the total plasma cholesterol value in both species consuming safflower oil diets, but not in those fed coconut oil. This decrease was essentially due to the significant decline in free cholesterol concentrations. The ratio of cholesterol esters to free cholesterol was maintained between 3.50–5.75 with a tendency for higher ratios in deficiency (Table 2).

The total plasma cholesterol concentration was much lower in the capuchins fed the safflower oil diets when compared to similar monkeys fed coconut oil, but the dietary fat did not affect the plasma cholesterol concentration of the cynamolgus. These differences reflected the doubling of cholesterol ester and free cholesterol in the capuchins fed coconut oil with essentially no change in the cynamolgus. These details supplement data previously reported for these species (18).

Assessment of the effect of dietary fat itself on plasma linoleate (18:2), arachidonate (20:4), and "other" cholesteryl esters, both as percent of total esters and in absolute quantities, proved interesting. Dietary safflower oil resulted in a twofold increase in the percentage of 18:2 in both species, but increased the absolute amount of 18:2 in the cynamolgus monkeys only. The maintenance of the 18:2 ester pool in the plasma of the capuchins fed coconut oil was associated with a doubling of the total cholesterol ester pool, an expansion resulting from a fourfold increase in "other" cholesteryl esters excluding 18:2 and 20:4 (Table 3). By contrast, coconut oil produced a 50% shrinkage in the absolute amount of 18:2 in the cynamolgus, but only doubled the "other" cholesteryl ester fractions resulting in no change in the total plasma cholesterol pool. The percentage of 20:4 was significantly increased by safflower oil, even though the absolute amount of this

TABLE 1
Effect of vitamin E deficiency on the amount and percent of free cholesterol esterified by plasma LCAT in two species of monkeys

| Species and diet | Cholesterol esterified, mg/dl per 24 hr | | | |
|---|---|---|---|---|
| | control | % FC | deficient | % FC |
| *Cynamolgus* | | | | |
| Coconut oil | 11.1 ± 1.1 (3) | 34 | 9.7 ± 0.8 (4) | 30 |
| Safflower oil | 8.3 ± 0.8 (3) | 29 | 4.8 ± 2.3 (2) | 28 |
| *Capuchins* | | | | |
| Coconut oil | 26.2 ± 5.3 (3) | 50 | 12.1 ± 7.5 (2) | 28 |
| Safflower oil | 9.3 ± 2.1 (2) | 34 | 4.5 ± 1.4 (4) | 25 |

Values represent mean ± SD. Numbers in parentheses indicate number of animals. Significant effects were found for fat ($P < 0.001$), vitamin E ($P < 0.001$), species ($P < 0.01$) and fat × species ($P < 0.01$).

TABLE 2
Effect of vitamin E deficiency on esterified, free, ratio of esterified to free, and total plasma cholesterol in two species of monkeys

| Species and diet | Plasma cholesterol, mg/dl | | | |
|---|---|---|---|---|
| | esterified | free | CE/FC ratio | total |
| *Cynamolgus* | | | | |
| Coconut oil | | | | |
| Control (3) | 129 ± 4 | 33 ± 3 | 3.91 | 162 ± 6 |
| Deficient (4) | 127 ± 9 | 32 ± 3 | 3.97 | 159 ± 10 |
| Safflower oil | | | | |
| Control (3) | 115 ± 30 | 29 ± 2 | 3.96 | 144 ± 32 |
| Deficient (2) | 97 ± 6 | 17 ± 2 | 5.70 | 114 ± 9 |
| *Capuchins* | | | | |
| Coconut Oil | | | | |
| Control (3) | 185 ± 35 | 52 ± 12 | 3.56 | 237 ± 47 |
| Deficient (2) | 206 ± 35 | 44 ± 10 | 4.68 | 250 ± 24 |
| Safflower | | | | |
| Control (2) | 104 ± 17 | 27 ± 3 | 3.85 | 131 ± 20 |
| Deficient (4) | 89 ± 16 | 18 ± 3 | 4.94 | 107 ± 17 |

Values represent mean ± SD. Numbers in parentheses indicate number of animals. For esterified cholesterol significant effects were found for fat ($P < 0.001$), species ($P < 0.01$), and fat × species ($P < 0.001$); for free cholesterol differences were noted for fat ($P < 0.001$), vitamin E ($P < 0.01$), species ($P < 0.05$), and fat × species ($P < 0.01$); for total cholesterol, fat ($P < 0.001$), species ($P < 0.01$), and fat × species ($P < 0.001$).

TABLE 3
Effect of vitamin E deficiency on the plasma concentration of linoleate, arachidonate, and "other" cholesterol esters in two species of monkeys

| Species and diet | Linoleate | | Arachidonate | | Other | |
|---|---|---|---|---|---|---|
| | % | mg/dl | % | mg/dl | % | mg/dl |
| *Cynamolgus* | | | | | | |
| Coconut Oil | | | | | | |
| Control (3) | 32.9 ± 7.7 | 42.4 ± 1.3 | 3.8 ± 1.0 | 4.9 ± 0.2 | 63.3 | 81.6 |
| Deficient (4) | 29.9 ± 3.4 | 38.0 ± 2.7 | 2.6 ± 1.0 | 3.3 ± 0.2 | 67.5 | 85.7 |
| Safflower Oil | | | | | | |
| Control (3) | 67.4 ± 1.6 | 77.5 ± 11.2 | 4.9 ± 1.1 | 5.8 ± 1.5 | 27.7 | 31.8 |
| Deficient (2) | 67.8 ± 1.1 | 65.8 ± 4.1 | 2.2 ± 0.8 | 2.1 ± 0.1 | 30.0 | 29.1 |
| *Capuchins* | | | | | | |
| Coconut Oil | | | | | | |
| Control (3) | 34.1 ± 2.7 | 65.1 ± 11.9 | 4.3 ± 1.1 | 8.0 ± 1.5 | 61.6 | 113.9 |
| Deficient (2) | 30.8 ± 3.0 | 63.4 ± 10.9 | 4.6 ± 0.9 | 9.5 ± 1.6 | 64.6 | 133.1 |
| Safflower Oil | | | | | | |
| Control (2) | 70.0 ± 3.7 | 72.8 ± 11.9 | 6.7 ± 2.8 | 7.0 ± 1.1 | 23.3 | 24.2 |
| Deficient (4) | 63.5 ± 2.6 | 56.5 ± 10.2 | 7.6 ± 1.1 | 6.8 ± 1.2 | 28.9 | 25.7 |

Values represent mean ± SD. Numbers in parentheses indicate number of animals. For percent linoleate esters a significant effect was found for fat ($P < 0.001$) only. For milligram linoleate, fat ($P < 0.01$) and fat × species ($P < 0.05$) were significant. For percent arachidonate, fat ($P < 0.01$), species ($P < 0.001$), fat × species ($P < 0.05$) and vitamin E × species ($P < 0.05$) were significant. For milligram arachidonate only species ($P < 0.01$) was significant.

ester was not affected by dietary fat. The capuchins maintained a significantly larger pool of arachidonate than did the cynamolgus.

An analysis of the fatty acid content of the plasma cholesteryl esters revealed a tendency for vitamin E deficiency to decrease linoleate esters, particularly remarkable in the capuchins fed the safflower oil diet (Table 3). In a similar fashion, the percentage and total concentration of arachidonate cholesteryl esters were depressed in the deficient cynamolgus monkeys fed either type of dietary fat. Arachidonate esters were not affected by vitamin E status in the capuchins. In effect, vitamin E deficiency resulted in a slight

increase in the percentage of "other" cholesteryl esters (Table 3).

## Discussion

These data suggest that vitamin E deficiency depressed plasma LCAT esterification, the effect being greatest in those monkeys fed the diet rich in polyunsaturated fatty acids and in the species that developed the most severe deficiency (14, 19). Since the requirement for vitamin E is related to the amount of polyunsaturated fat in the diet (2), it was not surprising that the monkeys fed the stripped safflower oil had the lowest plasma tocopherol values and were most severely afflicted.

Interpretation of the LCAT data must be made with caution since the activity measured during a 24-hour incubation period mainly reflects substrate concentrations and not necessarily LCAT activity in vivo which should be estimated from the initial rate (first hour) of esterification (15). The observation that LCAT activity was best correlated with the plasma concentration of free cholesterol is in keeping with similar observations in man and other species (20, 21). The highest LCAT activity was observed in the capuchins fed coconut oil, both in absolute amount and as a percentage of free cholesterol. This corresponds with the hypertriglyceridemia (18) and enhanced triglyceride synthesis (22) previously reported for capuchins fed coconut oil. Sodhi (23) and Nestel et al. (24) have revealed an association between hypertriglyceridemia and increased LCAT activity in man presumably resulting from an increased substrate transfer (lecithin and cholesterol) from triglyceride-rich lipoproteins to the high density lipoproteins where LCAT acts (15). It would appear that both substrates, free cholesterol and possibly 18:2 and 20:4 in the $\beta$-position of lecithin, were depressed in vitamin E-deficient monkeys fed safflower oil. An explanation for the decreased PUFA would be lipid peroxidation of their double bonds. In the safflower oil-fed capuchins this appeared as a slightly lower concentration of cholesterol linoleate, whereas the deficient cynamolgus demonstrated a significantly decreased percentage of arachidonate esters. It has also been demonstrated (25) that phosphatides can be damaged by in vitro peroxidation, although phosphatidylcholine appeared to be more stable than those containing a reactive amino group.

On the other hand, the concept of substrate depression by vitamin E deficiency was not supported by monkeys fed the coconut oil diet without vitamin E since plasma from these monkeys, at least the capuchins, tended to have depressed LCAT activity without real decreases in PUFA esters or free cholesterol. In addition, a recent study in rats confirming the depressed LCAT acitivity in vitamin E deficiency (26) revealed no change in serum free cholesterol, but did describe an unexplained rise in cholesterol esters. The cholesterol ester to free cholesterol ratio was also elevated in the deficient rats to approximately the same degree observed in these deficient monkeys. However, neither lecithin nor PUFA esters were measured in that study.

Another explanation for the depressed LCAT activity would be that tocopherol deficiency predisposes to peroxidative disruption of sulfhydryl sites on the LCAT enzyme itself. But, if the enzyme were damaged, one might expect an increase in plasma free cholesterol since a high concentration of free cholesterol has been demonstrated in familial LCAT deficiency (27). Since the free cholesterol concentration tended to be depressed in deficient monkeys, a definite explanation for the lowered LCAT activity cannot be ascertained without further investigation. One comes to a similar conclusion from the puzzling relationship of cholesterol esters, free cholesterol, and vitamin E reported for the tocopherol-deficient rat (25). As suggested in the study of LCAT activity in postmyocardial infarction (20), there may exist a number of unknown cofactors that influence the LCAT esterification process.

## References

1. TAPPEL, A. L. Vitamin E as the biological lipid antioxidant. Vitamins Hormones 20: 493, 1962.
2. HARRIS, P. L., AND N. D. EMBREE. Quantitative consideration of the effect of polyunsaturated fatty acid content of the diet upon requirements for vitamin E. Am. J. Clin. Nutr. 13: 385, 1963.
3. SCHWARZ, K., AND W. BAUMGARTNER. Kinetic studies on mitochondrial enzymes during respiratory decline relating to the mode of action of tocopherol. In: The Fat Soluble Vitamins, edited by H. F. DeLuca and J. W. Suttie. Madison: Univ. of Wisconsin Press, 1969, p. 317.
4. CHIO, K. S., AND A. L. TAPPEL. Inactivation of

ribonuclease and other enzymes by peroxidizing lipids and by malonaldehyde. Biochemistry 8: 2827, 1969.

5. CHIO, K. S., AND A. L. TAPPEL. Synthesis and characterization of the fluorescent products derived from malonaldehyde and amino acids. Biochemistry 8: 2821, 1969.
6. GLOMSET, J. A. The plasma lecithin-cholesterol acyltransferase reaction. J. Lipid Res. 9: 155, 1968.
7. MICKEL, H. S., AND E. L. FOULDS. The effect of hydrogen peroxide on serum lecithin-cholesterol acyltransferase activity. Lipids 5: 663, 1970.
8. MICKEL, H. S., E. L. FOULDS AND D. A. CLARK. Inhibition of the plasma lecithin-cholesterol acyltransferase reaction by hydrogen peroxide and peroxidized lecithin. Lipids 7: 121, 1972.
9. MICKEL, H. S., E. L. FOULDS, D. A. CLARK AND E. C. LARKIN. Effects of pure oxygen atmosphere in vivo on plasma lecithin-cholesterol acyltransferase reaction. Lipids 6: 740, 1971.
10. COOPER, R. A., AND C. L. GULBRANDSEN. The relationship between serum lipoproteins and red cell membranes in abetalipoproteinemia: Deficiency of lecithin:cholesterol acyltransferase. J. Lab. Clin. Med. 78: 323, 1971.
11. BIERI, J. G., AND R. K. H. POUKKA. Red cell content of vitamin E and fatty acids in normal subjects and patients with abnormal lipid metabolism. Intern. Z. Vitaminforsch. 40: 344, 1970.
12. KAYDEN, H. Abetalipoproteinemia. Ann. Rev. Med. 23: 285, 1972.
13. AUSMAN, L. M., K. C. HAYES, A. LAGE AND D. M. HEGSTED. Nursery care and growth of Old and New World infant monkeys. Lab. Animal Care 20: 907, 1970.
14. AUSMAN, L. M., AND K. C. HAYES. Vitamin E deficiency anemia in Old and New World monkeys. Am. J. Clin. Nutr. 27: 1141, 1974.
15. NORUM, K. R. The enzymology of cholesterol esterification. Scand. J. Clin. Lab. Invest. 33: Suppl. 137, 7, 1974.
16. HIRSCH, J., AND E. H. AHRENS. The separation of complex lipid mixtures by use of silicic acid chromatography. J. Biol. Chem. 233: 311, 1958.
17. ROSENTHAL, H. L., M. L. PELUKE AND S. BUSCAGLIA. A stable iron reagent for determination of cholesterol. J. Lab. Clin. Med. 50: 318, 1957.
18. COREY, J. E., K. C. HAYES, B. DORR AND D. M. HEGSTED. Comparative lipid response of four primate species to dietary changes in fat and carbohydrate. Atherosclerosis 19: 119, 1974.
19. HAYES, K. C. Pathophysiology of vitamin E deficiency in monkeys. Am. J. Clin. Nutr. 27: 1130, 1974.
20. SOLOFF, L. A., H. L. RUTENBERG AND A. G. LACKO. Serum cholesterol esterification in patients with coronary disease. Am. Heart J. 85: 153, 1973.
21. LACKO, A. G., H. L. RUTENBERG AND L. A. SOLOFF. Serum cholesterol esterification in species resistant and susceptible to atherosclerosis. Atherosclerosis 19: 297, 1974.
22. COREY, J. E., AND K. C. HAYES. Effect of diet on hepatic and intestinal lipogenesis in squirrel, cebus, and cynamolgus monkeys. Atherosclerosis 20: 405, 1974.
23. SODHI, H. S. Current concepts of cholesterol metabolism and their relationship to lecithin:cholesterol acyltransferase. Scand. J. Clin. Lab. Invest. 33: Suppl. 137, 161, 1974.
24. NESTEL, P. J., N. E. MILLER AND P. CLIFTON-BLIGH. Plasma cholesterol esterification in vivo in man. Scand. J. Clin. Lab. Invest. 33: Suppl. 137, 157, 1974.
25. BIDLACK, W. R., AND A. L. TAPPEL. Fluorescent products of phospholipids during lipid peroxidation. Lipids 8: 203, 1973.
26. TAKATORI, T., AND O. S. PRIVETT. Studies of serum lecithin-cholesterol acyltransferase activity in rat: Effect of vitamin E deficiency, oxidized dietary fat, or intravenous administration of ozonides or hydroperoxides. Lipids 9: 1018, 1974.
27. MARCEL, Y. L., AND C. VEZINA. Lecithin:cholesterol acyltransferase of human plasma. Role of chylomicrons, very low, and high density lipoproteins in the reaction. Scand. J. Clin. Lab. Invest. 33: Suppl. 137, 45, 1974.

**THE 12th WORLD CONGRESS OF THE INTERNATIONAL SOCIETY FOR FAT RESEARCH**

**MILAN (Italy) - 2-7 September 1974**

# SCIENTIFIC PROGRAMME

**THE CONGRESS IS UNDER THE HIGH PATRONAGE OF THE PRESIDENT OF THE REPUBLIC OF ITALY**

ORGANIZED BY SOCIETÀ ITALIANA PER LO STUDIO DELLE SOSTANZE GRASSE OF MILAN (ITALY)

**THE 12th WORLD CONGRESS OF THE INTERNATIONAL SOCIETY FOR FAT RESEARCH**

**MILAN (Italy) - 2-7 September 1974**

# ABSTRACTS OF PAPERS
# LIST OF PARTICIPANTS

Società Italiana per lo Studio delle Sostanze Grasse

(Italian Oil Chemists' Society)

**September 4, 1974**
**Afternoon - 3.00 - 6.15 p.m.**
**Section 4 - Room D**

## HUMAN PATHOLOGY

Chairmen: *Mickel H. S., Tettamanti G.*

| | |
|---|---|
| .00 - 3.15 p.m. | *Balta N., Mesinger V. and Cirja N. (Romania)* Disc electrophoresis of serum lipoproteins in some internal diseases. |
| .15 - 3.30 p.m. | *Böhm B., Gabor S., Zugravu E. and Kovats A. (Romania)* Lung and alveolar lipids in experimental silicosis. |
| 3.30 - 3.45 p.m. | *Balta N., Metz L., Capâlna S., Gioia G. and Cruceanu I. (Romania)* Lipemia and serum lipid fractions in atherosclerosis. Pathophysiologic implications. |
| 3.45 - 4.00 p.m. | *Cipollina Mangiameli G. (Italy)* The conditionings in the dismetabolic etiopathogenesis of fats. |
| 4.00 - 4.15 p.m. | *James J. A., Bolton C. H. and Read A. E. (U.K.)* Experimental production of lipoprotein X (LPX) in the absence of obstructive jaundice. |
| 4.30 - 4.45 p.m. | *Mickel H. S., Hill P. L. and Hayes K. C. (USA)* Depression of the plasma lecithin-cholesterol acyltransferase reaction in vitamin E deficiency. |
| 4.45 - 5.00 p.m. | *Mickel H. S., Horbar J. L. and Stamets J. (USA)* Peroxidized arachidonic acid effect on human platelet aggregation. |
| 5.00 - 5.15 p.m. | *Hausdörfer J., Heller W., Junger H. and Oldenkott P. (W. Germany)* Modification of fat metabolism following experimental head injury. |
| 5.15 - 5.30 p.m. | *Junger H., Heller W. and Hausdörfer J. (W. Germany)* Studies of fat metabolism in banked blood. |
| 5.30 - 5.45 p.m. | *Gass G. H., Allaben W. T., Brown H. J., Liu S. L. and Bentrley M. J.* The endocrine role of dietary lipids in mammary tumor formation. |
| 5.45 - 6.00 p.m. | *Van den Berg D (Netherlands)* Problems in initiating n-3 Deficiency in rat brain. |
| 6.00 - 6.15 p.m. | *Karageosian C. G. (USSR)* Enzymatic systems involved in initial steps of phosphatidogenesis in normal and pathological states. |



### 81

DEPRESSION OF THE PLASMA LECITHIN-CHOLESTEROL ACYLTRANSFERASE REACTION IN VITAMIN E DEFICIENCY.

*H.S. Mickel, L. Hill and K.C. Hayes.*
Children's Hospital Medical Center & Harvard School of Public Health Boston, Massachusetts, U.S.A.

Vitamin E deficiency was studied in two species of monkeys; a reduction in the extent of esterification by the plasma lecithin cholesterol acyltransferase reaction occurred in both species. The reduction was greatest in animals fed a diet rich in polyunsaturated fat and stripped of vitamin E content. A decrease in the concentration of circulating polyunsaturated fatty acid cholesteryl esters occurred in the vitamin E deficient animals. Since the plasma lecithin-cholesterol acyltransferase reaction has been shown to be dependent upon sulfhydryl sites on the enzyme, it is proposed that the observed reduction in esterification of cholesterol is due to alteration of these sulfhydryl sites. A reduction in the plasma lecithin-cholesterol acyltransferase reaction has been reported by us to occur during exposure *in vivo* to a pure oxygen atmosphere, a condition known to predispose to lipid peroxidation.

### 82

PEROXIDIZED ARACHIDONIC ACID EFFECT ON HUMAN PLATELET AGGREGATION.

*H.S. Mickel, J.L. Horbar and J. Stamets.*
Children's Hospital Medical Center, Boston, Massachusetts, U.S.A.

The addition of peroxidized arachidonic acid to suspension of human platelets in buffer or to platelet-rich plasma results in abrupt platelet aggregation. Unperoxidized arachidonic acid effects a much slower rate of aggregation, comparable to that described for saturated fatty acids.
High concentrations of adenosine and prostaglandin $E_1$ blocked the effect of peroxidized arachidonic acid-induced platelet aggregation in platelet-rich plasma. Similar, prior treatment *in vivo* with high doses of aspirin inhibited the *in vitro* platelet aggregation response to peroxidized arachidonic acid.
Peroxidized arachidonic acid can be adsorbed onto platelet aggregates *in vitro* without desctruction of the lipid peroide.
Peroxidized eicosatrienoic acid and peroxidized linoleic acid had no appreciable effect on platelet aggregation.

**September 5, 1974**
Afternoon - 3.00 - 6.45 p.m.
Section 14 - Room D

## AUTOXIDATION AND ANTI-OXYDANTS

Chairmen: *Graille J., Fukuzumi K.*

3.00 - 3.15 p.m. *Fukuzumi K., Ikeda N. and Egawa M. (Japan)*
New antioxidants and synergists for the autoxidation of fatty acid esters.

3.15 - 3.30 p.m. *Fromberz P. (W. Germany)*
Lipoid pH- indicator in monolayers of lipids and proteins.

3.30 - 3.45 p.m. *Fricker A. (W. Germany)*
Influence of thermal treatment on the lipid compounds of spinach.

3.45 - 4.00 p.m. *Tirzit G. D. and Dubur G. Ya. (URSS)*
« 1,4 dihydropyridine derivatives ». A new class of lipid antioxidants.

4.00 - 4.15 p.m. *Allen J. C., Crook E. M. and Farag R. S. (U.K.)*
The metal-catalysed oxidation of lipids in aqueous emulsion and the influence of specific phospholipids on the reaction.

4.15 - 4.30 p.m. *El-Zeany P. A. and Pokorny J. (Czechoslovakia)*
Brown products of oxidized fish oil esters - Protein interaction.

4.45 - 5.00 p.m. *Meara M. L. and Weir G. S. D (U.K.)*
The effect of β-carotene on the stability of palm oil.

5.00 - 5.15 p.m. *Graille J., Guillaumin R. and Naudet M. (France)*
Comparison of various chromatographic methods in the field of isolation and study of oxidative and thermal alteration products of fats.

5.15 - 5.30 p.m. *Paled M. and Letan A. (Israel)*
Studies of cottonseed oil exposed to conditions of frying.

5.30 - 5.45 p.m. *Mickel H. S., Horbar J. L. and Gerwitz M. (USA)*
Autoxidation of polyunsaturated fatty acid methyl esters in oxygen mixture atmospheres.

5.45 - 6.00 p.m. *Pokorny J., Tai P., and Janicek G. (Czechoslovakia)*
Autoxidatives browning of phospholipids.



### 180

AUTOXIDATION OF POLYUNSATURATED FATTY ACID METHYL ESTERS IN OXYGEN MIXTURE ATMOSPHERES.

*H.S. Mickel, J.L. Horbar and M. Gewirtz.*
Children's Hospital Medical Center, Boston, Massachusetts, U.S.A.

Autoxidation of methyl linoleate *in vitro* by exposure at 50 C to oxygen mixture atmospheres is greater in a 80% helium- 20% oxygen (vol. %) atmosphere than in an 80% argon- 20% oxygen atmosphere. In turn, autoxidation is greater in an 80% argon- 20% oxygen atmosphere than in an 80% nitrogen- 20% oxygen atmosphere. Comparable findings occurred with peroxidation of methyl linolenate. It is proposed that the observed phenomenon is attributable to a quenching gas effect or to physical interaction of the inert gas with the unsaturated lipid.

### 181

AUTOXIDATIVE BROWNING OF PHOSPHOLIPIDS.

*J. Pokorný, P. Tài and G. Janíček.*
Dept. of Food Chemistry, Prague Institute of Chemical Technology, Prague, Czechoslovakia.

Phosphatidyl ethanolanine and phosphatidyl choline isolated from egg yolk were oxidized in thin films at 20 - 100°C in dark and under excess of oxygen. At the same degree of oxygen absorption phosphatityl choline became only slightly coloured but phosphatidyl ethanolamine turned dark brown. Brown products were formed through colourless intermediates by decomposition of peroxides. The brown products were fractionated by chromatography on DECAE-cellulose and on Sephadex LH-20 and spectral characteristics of the fractions were determined. One fraction was rich in nitrogen, on contrary, some fractions were nitrogen-free. Brown products were saponified and the acidic fraction separated by TLC on silicic acid. Less polar products were light in colour, contained only traces of nitrogen, a carbonyl and a hydroxyl group. The brown less mobile fraction was rich in nitrogen and oxygen and contained an imino group. The acetylation or treatment with phthalic anhydride of phosphatidyl ethanolamine did not prevent oxidative browning but the formaldehyde-treated product did not turn brown. Mitures of ethyl linoleate and ectadecyl amine became yellow only slowly during autoxidation. All fractions of brown products were bleached by treatment with hydrogen peroxide.

### 182

STORAGE OF CEREAL PRODUCTS AND OXIDATION OF FLOUR LIPIDS.

*P. Scheffeldt.*
Agrikulturchem. Institut ETHZ, Zürich, Switzerland.

During the storage of certain cereal products (i.e. babyfoods, bread) the development of off-flavor is observed. This off-flavor is probably formed by the oxidation of flour lipids. White wheat flour contains only about 1.5% lipids. However this small quantity can be responsible for the deterioration of a number of processed cereals.
Storage experiments were carried out on wheat flour, roller dried wheat flour and bread by following the changes in lipid composition (t.l.c. and g.l.c.). Wheat flour showed an increase in free fatty acids and a decrease in sterolesters, while the other lipid components showed practically no changes. In roller dried wheat flour and bread a decrease in free fatty acids was the most obvious change while new bands appeared in t.l.c. It was been shown by GRAVELAND that the free fatty acids are readily oxidised in heat flour/water systems by flour lipoxygenase to

LA RIVISTA ITALIANA
DELLE
SOSTANZE GRASSE

Stazione Sperimentale
per le Industrie degli Oli e dei Grassi

20133 MILANO 29.12.1976
VIA GIUSEPPE COLOMBO, 79
TELEFONO 23.61.051/2/3/4

/lp

Mr. HUBERT S. MICKEL, M.D.
Department of Neurology
Children's Hospital Medical Center
BOSTON, Massachusetts 02115 USA

Dear Sir,

with regard to your kind letter of November 13, 1976 we inform you that the cost for 200 additional reprints of your article "Effect of inert gases and nitrogen on the autoxidation of cis and trans polyunsaturated fatty acid methyl esters" will be 60.000 Liras + postage.

Awaiting your reply we are,

Yours sincerely
The Director
(Ing. Cesare Carola)

IUB

# TENTH INTERNATIONAL CONGRESS OF BIOCHEMISTRY

Hamburg (Federal Republic of Germany)
July 25 to 31, 1976

## ABSTRACTS

## 13-8-194 THE EFFECT OF UREMIC PEPTIDE ON INSULIN-STIMULATED $Mg^{++}$-ATPase ACTIVITY OF HUMAN ADIPOCYTES

W. Lutz

Department of Medical Analytics, Institute of Internal Medicine, 90-549 Łódź /Poland/

From uremic plasma there was isolated a peptide with strong alkaline properties, of which presence has not been found in plasma of healthy men. This uremic peptide, added to incubative medium containing shadows of human adipocytes, inhibited stimulative effect of insulin on $Mg^{++}$-ATPase activity. Inhibitory effect produced by the uremic peptide was greatly decreased after introduction of albumin to incubative medium. The uremic peptide had no direct influence on $Mg^{++}$-ATPase activity.

## 13-8-195 THE PHYSICAL, CHEMICAL AND IMMUNOLOGICAL PROPERTIES OF AN ADULT HUMAN KIDNEY PLASMINOGEN ACTIVATOR

Maciag, T., Pye, E. K. and Iyengar, M. R.
Department of Animal Biology, Laboratories of Biochemistry, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19174 U.S.A.

A plasminogen activator was isolated from the tissue culture growth media of adult human kidney (AHK) tissue culture cells. Purification of the plasminogen activator by existing affinity chromatography methods resulted in specific activities greater than 50,000 CTA units per mg protein. Gel exclusion chromatography and SDS disc gel electrophoresis studies showed that the AHK plasminogen activator possesses a molecular weight of 55,000 daltons. Ampholine disc gel electrophoresis of the AHK plasminogen activator and urokinase (Serano Laboratories) generated an isoelectric point of pH 8.75 for both enzymes. Kinetic analysis of the AHK plasminogen activator using the CTN assay yielded a Km (CTN) value of $4.5 \times 10^{-4}$ and a Vm(CTN) of $8.11 \times 10^{-10}$ moles of p-nitrophenoxide released per second per CTA unit. Competitive inhibition kinetics were observed with the ligands BAA and TAME. Inhibition constants of $1.0 \times 10^{-4}$ and $3.8 \times 10^{-4}$ M were obtained for BAA and TAME respectively. Using the casein assay, we observed a pH maximum of 8.9 for the AHK plasminogen activator. Rabbit antiserum was prepared against the purified AHK plasminogen activator and upon double immunodiffusion, lines of identity were observed between the AHK and human urine plasminogen activators. Double immunodiffusion experiments using antiserum against urokinase also resulted in lines of identity between the two human plasminogen activators. The AHK plasminogen activator was observed to be immunologically similar but not identical to a plasminogen activator purified from the tissue culture growth media of adult pig kidney cells.

## 13-8-196 AUTOXIDATION OF CIS AND TRANS POLYUNSATURATED FATTY ACIDS IN OXYGEN AND INERT GAS MIXTURE ATMOSPHERES

H.S. Mickel, R. Ansari, and J. Horbar

Children's Hospital Medical Center, Boston, MA 02115 U.S.A.
Harvard Medical School, Boston, MA 02115 U.S.A.
Wrentham State School, Wrentham, MA 02093 U.S.A.

Autoxidation of methyl linoleate and methyl linoleidate were studied by coating a thin film of the methyl ester onto the wall of a swirling flask, incubated at 50 C, and exposed to an atmosphere of approximately 20% oxygen with the balance an inert gas, carbon dioxide, or nitrogen. Gases present in the atmosphere other than oxygen alter the rate of the autoxidation reaction with fatty acids containing double bonds in the cis configuration. The greatest autoxidation occurs in a helium-oxygen atmosphere, less occurs in an argon-oxygen atmosphere, and still less in a nitrogen-oxygen atmosphere. Little difference is noted in the autoxidation reaction in these oxygen mixture atmospheres with fatty acids containing double bonds in the trans configuration. Monolayer studies of autoxidation were carried out by incubating fatty acids coated onto silica gel by the method of Mead and Sgoutas. Differences in autoxidation of cis and trans fatty acids in oxygen mixture atmospheres were again observed.
The observations indicate an interaction between the inert gases and cis double bonds, but not trans double bonds. Such an interaction might account for the phenomenon of inert gas narcosis, the high pressure neurological syndrome, as well as other physiological phenomena observed under hyperbaric conditions.

## 13-8-197 VERY LOW DENSITY LIPOPROTEIN (VLDL) COMPOSITION IN FAMILIAL HYPERLIPOPROTEINAEMIA

I.C. Ononogbu and B. Lewis

Department of Chemical Pathology, Royal Postgraduate Medical School, DuCane Road, England.

VLDL concentration varies substantially in the different hyperlipoproteinaemic states. In the rare entity type III hyperlipoproteinaemia, VLDL composition is known to be abnormal, but in most forms of human hyperlipidaemia this has not been extensively studied. The cholesterol and triglyceride contents of VLDL, isolated by precipitation with sodium dodecyl sulphate was measured in 19 controls and 26 patients with familial hyperlipoproteinaemia. In the controls, cholesterol to triglyceride ratio was 0.32-0.01 (S.E.M.). The VLDL lipid ratio was not significantly different in patients with raised low density lipoprotein (LDL) levels (0.29-0.03, n=6). In type III the ratio was confirmed to be high (0.51-0.03, n=4) ($p < 0.001$). In 8 patients with raised levels of LDL and VLDL (type IIb), VLDL composition was also abnormal (0.38-0.02) ($p < 0.05$). Those with high VLDL levels (type IV) had a significantly ($p < 0.01$) low ratio (0.24-0.02, n=8). In this condition, enhanced endogenous triglyceride transport may be mediated by increased triglyceride content of VLDL as well as increased number of circulating VLDL molecules.

## 13-8-198 THE RELATIONSHIP BETWEEN THE C1T SUBCOMPONENT OF C1T AND AMYLOID P-COMPONENT.

R. H. Painter, P. A. Taylor, & T. Hofmann
Department of Biochemistry, University of Toronto, Ontario. M5S 1A1, Canada.

C1t is a 9.5 S al glycoprotein isolated from human serum by affinity chromatography on IgG sepharose. It forms part of the calcuim linked macromolecular C1 complex. The molecular weight of C1t has been shown by sedimentation equilibrium analysis to be 233,000 and to be composed of 10 identical subunits of molecular weight 23,000 arranged in the form of 2 pentagons joined at one of their faces. This protein appears in electron micrographs with negative staining as pentagonal figures 85 A° in diameter which assemble as rods of varying length. The electron micrographs show a strong resemblance to those of P-component, a 9.5 S al plasma glycoprotein purified from human amyloid. Indeed C1t and P-component have been shown to react identically with each other's antisera. Both primary and secondary amyloid may be stained with antisera to C1t. The amino acid composition and sequence studies indicate marked similarities between C1t and P-component although some significant differences between the sequences were evident. The N-terminal amino acid sequence of C1t shows about 50% homology with the N-terminal sequence of C reactive-protein, a phosphoryl-choline binding protein also comprised of 23,000 molecular weight subunits. The relationships and differences between these three proteins will be discussed.

Supported by MRC of Canada

## 13-8-199 THE EFFECT OF EARLY NUTRITION ON LIPID METABOLISM OF THE ADULT RATS

M. Palkovič, N. Škottová

Institute of Experimental Endocrinology Slovak Academy of Sciences, Bratislava, Czechoslovakia

In order to demonstrate the possible late consequences of early nutrition on lipid metabolism in the rats, we studied the level of blood lipids /triglycerides, NEFA, cholesterol, phospholipids and lipoproteins /and activity of malic enzyme /L-malate:NADP decarboxylating oxidoreductase, EC 1.1.1.40/ and ATP citrate lyase /EC 4.1.3.8/ in the liver of the adult rats /6months old/ fed the usual laboratory diet with various content of cholesterol between 18-to 30 postnatal days. We did not observed any metabolic disturbance under resting conditions. However, after exposition of these old animals to high cholesterol diet, the rats with lower early intake of cholesterol had higher level of blood cholesterol, higher concentration of beta lipoprotein cholesterol and higher activity of liver enzymes. These results suggested important role of early nutrition on lipid metabolism.

AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS
— INCORPORATED —

ROBERT A. HARTE, EXECUTIVE OFFICER
9650 ROCKVILLE PIKE
BETHESDA, MARYLAND 20014

PHONE:
AREA CODE 301
530-7145

Serial 6-406
2 April 1976

Dr. Hubert S. Mickel
Children's Hospital Medical Center
Boston, Massachusetts 02115

Dear Colleague:

It is a pleasure to advise you that the funds whose receipt was anticipated when you were recently notified of the actions of the Travel Award Committee have materialized and you are one of 201 awardees receiving travel grants in the amount of $500. Herewith you will find the award to be used to facilitate your participation in the 10th International Congress of Biochemistry, convening in Hamburg, Federal Republic of Germany, 25-31 July 1976.

We hope that your participation in the Congress will help to make for effective U.S. representation at the meeting, to advance biochemical science, and to contribute to your personal and professional advancement as well.

You are reminded that the Federal Agencies as donors of the bulk of the funds being distributed have specified that, wherever possible, individual awardees are expected to use U.S. flag carriers, particularly for the transatlantic portions of their travel.

Please be sure to notify this office, as promptly as possible, in the event that circumstances change so that you will not need or cannot use this travel award. We have a substantial standby list of highly qualified biochemists who would appreciate the opportunity to use funds released by any of the present list of awardees. Your prompt return of the money awarded you, in the event your plans change, will be much appreciated by the individuals on that standby list.

You will also find herewith a certification of attendance at the Congress, which you are requested to complete and return as promptly as possible following the conclusion of the Congress. Attempts will be made to correlate your answers in the hope that they will be useful in supporting future proposals for travel award funds. The responses of the recipients of travel awards to the Stockholm Congress were of significant value in obtaining funds for the present program.

All good wishes for a pleasant and profitable stay in Hamburg.

Yours sincerely,

Robert A. Harte

RAH/mak
Enclosures: 2

Proposal for subcontract with Department of Chemistry, Harvard

# INTERACTION OF LIPIDS WITH INERT GASES

Mickel and coworkers have observed remarkable differences in the extent of autooxidation of cis and trans lipid molecules in various oxygen-atmosphere mixtures. For the cis-lipid, autooxidation for a fixed volume percent of oxygen is greater for a helium-oxygen atmosphere than an argon-atmosphere, which in turn is greater than in a nitrogen-oxygen atmosphere. For the trans-lipid, the extent of autooxidation is not affected by the gas other than oxygen. The isomeric lipid molecules differ only in the configuration of a pair of double bonds. The molecule referred to as "cis" is cis-9, cis-12-octadecadienoic acid and the molecule referred to as "trans" is trans-9, trans-12-oxtadecadienoic acid. Thus the marked differences seen in the autooxidation strongly suggest that there may be a specific interaction of the inert gas (helium, argon, or nitrogen) with the carbon-carbon double bonds. Such an effect would have much fundamental interest and might elucidate many unexplained in vivo phenomena involving autooxidation in the presence of artificial atmospheres.

Discussions with Dr. Mickel have led us to propose a collaborative study in which we seek to examine the interaction of these lipid molecules and simpler model compounds with the rare gases and other inert molecules to look for specific binding at the double bonds. This work would take advantage of molecular beam and mass spectrometric methods to examine the molecular interactions. Three experimental programs are planned. (1) A gas flow kinetic apparatus would be constructed to repeat under more controlled conditions the original experiments of Mickel and coworkers, and to extend those to Xenon and

other molecules for which we suspect the effect might be even more pronounced. (2) A molecular beam study would be carried out in which the lipid is mixed in a supersonic nozzle source with various inert gases and the mass spectrum examined for evidence of complex formation. (3) In another molecular beam apparatus, beams of the lipid or model compounds would be collided with beams of the inert gases and the scattering pattern analyzed to derive information about the potential energy function for the interaction.

Our laboratory has been devoted to molecular beam studies for the past seventeen years and has developed many of the basic methods which allow direct observation of molecular interactions. The studies of the lipid systems would be carried out by a postdoctoral fellow, Dr. Will Lee, and by two graduate students, Mr. Kit Bowen and Mr. Jonathan Sokol. All would be involved in the three stages of the work. Dr. Lee has much experience with molecular beam methods appropriate to this study. In particular, he has just concluded a major research investigation of the complex formation in supersonic beams of water, ammonia, and methanol; this involved the analysis of mass spectra patterns for clusters of these molecules formed in the supersonic expansion. For example, he could observe clusters of water or ammonia containing twenty or thirty molecules. Messrs. Bowen and Sokol have also had much experimental experience with beam techniques.

We would expect the design and construction of the kinetic apparatus for the stage (1) study would be completed within 2 months and those experiments would then be pursued under the direct supervision of Dr. Mickel. The first step in the molecular beam studies (2) and (3) could be started

immediately. This will involve a study of the vaporization properties and the mass spectroscopic fragmentation pattern of the cis and trans lipid molecules. Unless unforeseen difficulties intervene, we would expect that within 6 months the beam studies would have progressed far enough to demonstrate whether a specific interaction of the inert gases with double bonds exists in these compounds.

Subcontract with the Department of Chemistry, Harvard University

PROGRESS REPORT

(August 15 to December 1, 1976)

The kinetic apparatus has been constructed and three sets of preliminary experiments have been carried out to check the original results of Mickel and coworkers. Several runs have been made for $He/O_2$, $Ar/O_2$ and $N_2/O_2$ mixtures interacting with the cis lipid. Satisfactory experimental reproducibility has not yet been achieved. However, we hope soon to obtain marked improvement by adding suitable normalization procedures and we have now almost operational a gas chromatography unit for this purpose.

A "solubility meter" has been designed and constructed to determine Henry's law constants for interaction of gases with lipids. These measurements will be carried out within the next month.

Molecular beam studies of the interaction of Ar, Kr, and $N_2$ with cis-and trans-butene have been carried out. This simple system was examined to provide a reference model for comparison with the similar experiments being prepared for the lipid systems. In these experiments with butene, the olefin vapor at a partial pressure of a few torr was mixed with an excess of the interacting gas at a pressure up to 1000 torr and expanded through a pin-hole nozzle into the high vacuum system. The composition of the resulting molecular beam was examined by mass spectroscopy. The experiments were repeated for a range of source temperatures and mixing ratios of the gases. The mass spectra show that the butene molecules interact strongly to form substantial yields of butene dimers and even higher polymers but there is no evidence for significant formation of complexes between the butene and Ar, Kr, or $N_2$. Of course, butene differs drastically from the lipid molecules of interest, which have a pair of double bonds separated by a $CH_2$ group. However, this result already serves to establish that any hypothesis which attempts to explain the auto-oxidation behavior of the lipids in terms of formation of a complex with the inert gas must consider more than a possible complex with a single double bond.

Hubert S. Mickel, M.D. Effects of Inert Gases on Lipids

## SUMMATION

A phenomenon was observed during the period of this contract which has high potential usefulness for the Navy in its understanding of the effects of atmospheres on biological systems. There appears to be an interaction between various gases and polyunsaturated lipids having two or more bonds in cis configuration, and a mothylene carbon between the double bonds, as in linoleic acid. There were limitations as to the kind of information that might be proferred regarding a mechanism for this interaction, should this investigation be kept within the lipid neurochemistry laboratories at the Wrentham State School and Children's Hospital Medical Center. For this reason, a collaborative undertaking was sought and obtained with Professor Dudley Herschbach, of the Department of Chemistry at Harvard University, where the technique of molecular beam spectroscopy was available to explore this topic. The contract has been renewed through the Department of Chemistry with a new contract number, N00014-77-C-0216, effective March 15, 1977. Definitive information regarding the nature of the observation is expected by transferring the investigation to the Department of Chemistry.

There are several topics which papers are expected to be submitted for publication besides those listed in this report. An alteration of arachidonic acid levels in the liver in children's dying with Reye's syndrome was observed and a manuscript on this topic is being prepared with speculation regarding the mechanism of the polyunsaturated fatty acid reduction and its role in the disease process. Further observations on the effects of various peroxidized polyunsaturated fatty acids on platelet aggregation were made, reported at the International Society for Fat Research Meetings in Milan, Italy, in September, 1974, but not yet prepared for publication. On each of these, as well as other topics, when submitted for publication, acknowledgement will be made to the ONR and to this contract.

The biological aspects of peroxidized lipids are now being investigated in a collaborative undertaking with Dr. K. C. Hayes of the Department of Nutrition, Harvard School of Public Health, and the instrumentation and laboratory equipment not brought to the Department of Chemistry have been transferred to these laboratories. Funding for this aspect of the work is not currently supported by the Office of Naval Research.