Antioxidants' protection against peroxidation

Newspapers, popular magazines, and TV are all focussing on health implications of lipid peroxidation and on nutrient antioxidants to prevent oxidative damage to metabolic processes and oxidative damage to human tissues. Headlines abound: “Studies find use of vitamin E reduces risk of heart disease; “Carotenoids may help decrease cataract risk; Selenium, essential trace mineral, part of one of the antioxidant enzymes that complements vitamin E to fight cell damage.”

Scientists working in this area have found oxidative lipid damage in aging and exercise and the following chronic diseases: cancer, ischemic heart disease, circulatory conditions, cataracts, and air pollution (1). Of these conditions, our experience has been mainly with air-pollution damage, oxidative damage caused by ozone, nitrogen dioxides and halogenated hydrocarbons. We found that vitamin E protects experimental animals from oxidative air pollution.

The fields of lipid peroxidation and vitamin E are in explosive growth. For example, publications on lipid peroxidation in animal tissues in vivo started with some of our studies around 1960. Now there are 1,200 publications per year on this subject. Vitamin E activity was discovered on our Berkeley campus. We entered the field in 1950 and were able to do some of the early studies in the next two decades. The volume of publications per year is now 800.

Concerning practical applications of this developing knowledge, there are few overall summaries. Approximately 100 million people in the United States take vitamins, with more than one-third taking vitamin E and over one-third taking vitamin C. Global vitamin E production is estimated at 20 million kg, of which 95% goes into animal feeds. The U.S. market is estimated at $100 million for β-carotene and over $300 million for vitamin E. From the above information and present knowledge of beneficial health effects of anti-oxidogenic nutrients, some scientists in this area approximate that millions of person years are being saved. A study by Pracno (Reston, Virginia) suggests that antioxidant vitamin use by the U.S. population could save $8.7 billion in health-care costs annually by reduction of cardiovascular disease and some cancers.

The knowledge of oxidative damage and antioxidants might be though of as a pyramid of knowledge with chemistry and biochemistry as the basic information. Most of the biochemical knowledge concerns the effects of disease and the biochemistry of tissues. At the apex of the knowledge pyramid is applications for people. Epidemiologic data of antioxidant protection is the gold standard of the field near the applications-for-people portion of the pyramid. Between the basic information and epidemiologic data, I would put experimental animal studies of oxidant damage and antioxidant protection.

There are certain questions important to the field and related to the research done in our laboratory: (a) the importance of animal testing of the effectiveness of antioxidants; (b) the quantitation of antioxidant protection; (c) the achievement of maximum antioxidant protection; and (d) the antioxidant strength of β-carotene.

In the human situation, there are various dietary intakes of antioxidant nutrients and diverse detrimental consequences of oxidative damage leading to aging and diseases. An animal model of this situation consists of using a refined diet with defined addition of dietary antioxidants. After deposition of the antioxidants in the animal's tissues—perhaps taking several weeks to a month—accelerated oxidation can be induced. The ensuing oxidatively damaged products can be measured by analytical procedures such as the thiobarbituric acid assay (TBARS) and heme protein oxidation.

Work on oxidative damage to living animals and animal tissue has defined lipid peroxidation as a major oxidative and deteriorative process (1). During the past 50 years, vitamin E has been found to be the primary lipid antioxidant of nature (2), a fact of major importance to the lipid biochemist. Other antioxidants of some significance include β-carotene, containing a hydrogen more difficult to extract than that of vitamin E for antioxidant protection. Further, when a hydrogen is abstracted from β-carotene, this antioxidant cannot be

(continued on page 780)
functionally regenerated as can vitamin E. Therefore, compared to the activity of vitamin E, \( \beta \)-carotene is a weak antioxidant. Another important antioxidant group is coenzyme Q in the reduced form. Coenzyme Q is not only in the mitochondrial electron transport chain but also in other membranes and lipids of the body and consequently can act as a chain-breaking antioxidant. Another important nutrient for antioxidative activity is selenium, which becomes incorporated as the active site of the enzyme selenium glutathione peroxidase (3). This enzyme reduces and thereby inactivates hydrogen peroxide and lipid peroxides which are oxidative compounds.

In addition to early studies on vitamin E, members of our laboratory have investigated coenzyme Q as an important lipid antioxidant and also selenium's incorporation into animal tissue to form the enzyme selenium glutathione peroxidase.

**Antioxidants in tissues**

In these dietary studies to investigate antioxidant protection against oxidation, control rats and chicks were fed vitamin E- and selenium-deficient diets. The basal diet for the rats contained 10% tocopherol-stripped corn oil, 30% torula yeast, 56% sucrose, and 4% minerals and vitamins. The basal diet for chicks contained 5% tocopherol-stripped corn oil, 20% soy protein, 68% starch, dextrose, etc., and 7% minerals and vitamins. The antioxidants to be tested for protection against oxidative damage were added to the basal diets and fed to the rats or chicks. After the animal has incorporated the elements of the diet and the antioxidants, tissues are taken from the animal and kept in metabolic integrity as they are shaken in air with or without an oxidizing agent. After a period of time, tissues are analyzed by methods such as TBARS, which measures lipid peroxidation products, and a test for oxidized heme proteins.

An example of this type of research addresses the quantifying of antioxidant protection and the question of the strength of \( \beta \)-carotene as an antioxidant (4). The protocol is shown in Table 1. The protocol involves 0 vitamin E with the addition of increasing amounts of \( \beta \)-carotene fed to chicks as shown under “Dietary.” Another part of the experiment uses 50 IU vitamin E per kilogram of diet fed to chicks with increasing doses of \( \beta \)-carotene. The dietary amount per kilogram of animal diets is similar to the dietary amounts of the constituent antioxidants expressed as units or milligrams per day in human diet. The analyses of the chick liver show that \( \beta \)-carotene is incorporated as a function of its dietary concentration. However, at the highest amount of \( \beta \)-carotene in the chicks' diet, there is a mutual interference in the hepatic absorption of vitamin E and \( \beta \)-carotene, with both being reduced. Particularly, the poor hepatic absorption of vitamin E results in increased lipid peroxidation in liver as measured by TBARS. Figure 1 records, via decreasing TBARS, the protection to liver slices offered by increased amounts of dietary vitamin E and \( \beta \)-carotene in a biological system wherein iron is used to induce lipid peroxidation. Figure 1 indicates lowered concentration of TBARS in

Table 1

<table>
<thead>
<tr>
<th>Hepatic vitamin E and ( \beta )-carotene concentration</th>
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<tbody>
<tr>
<td><strong>Diet</strong></td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>EO, CO</td>
</tr>
<tr>
<td>EO, C30</td>
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<tr>
<td>EO, C100</td>
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<tr>
<td>E50, CO</td>
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<tr>
<td>E50, C30</td>
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<tr>
<td>E50, C100</td>
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Figure 1. Effect of diet and iron on the TBARS produced by liver slices in 1-hr incubation. (Bars show TBARS means ± standard deviation. TBARS with different letters within the same iron concentration are significantly different at \( P \leq 0.05 \).)
Polyunsaturated lipids are oxidized by tissue activator enzymes (P450) which are induced by antioxidants (vitamin E) and glutathione. The reaction is controlled by the peroxidizability of the polyunsaturated fatty acids (PUFA) and inhibited by antioxidants such as vitamin E. The simulation model also incorporates the autoxidation cycle and the reduction by selenium glutathione peroxidase of the hydroperoxides formed in the oxidation of lipids. Finally, the model produces TBARS reactants, the measurement of compounds formed in the oxidation reaction. Although empirical, this model incorporates some of the main parameters and relationships in the scientific literature and is a dynamic working model of chemical reactions that ensue in tissues. This is the first simulation model of lipid peroxidation, but further study and more detailed models are needed.

For the experimental results shown in Figure 1, a simplified model can be developed which presents the main determining parameters of the oxidation reaction, vitamin E and β-carotene:

\[ \text{TBARS} = \text{lipid peroxidation components} \times \log(\text{Fe}) + \text{basal lipid peroxidation vitamin E} + 0.5 \beta\text{-carotene} \]

“Lipid peroxidation components” is a composite of the factors involved in the reaction which are not otherwise expressed in the equation. In the model, β-carotene is assigned a strength of 0.5 compared to that of vitamin E. However, low concentration of β-carotene in the tissue diminishes its effect as a viable antioxidant. A correlation for the experimental results in a particular study, with the results predicted by a simulation model of the reaction, can test the validity of the simulation model in its highly aggregated form as a good model of the actual experimental results. In this experiment, the correlation between simulated TBARS and experimental TBARS is very good, \( R = 0.68, P = 0.001 \).

**Heme protein oxidation and multitioxidants**

There is need for new methods of measuring tissue oxidation and the oxidative processes in animals that are being studied. We have developed a new method of measuring heme pro-
Table 2
Addition of antioxidant nutrients to basal diet

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>Diet group</th>
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<tbody>
<tr>
<td>Vitamin E</td>
<td>B 0 0 0</td>
</tr>
<tr>
<td>Selenium</td>
<td>B 0 0 0</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>B 0 0 0</td>
</tr>
<tr>
<td>Coenzyme Q_{10}</td>
<td>B 0 0 0</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>B 0 0 0</td>
</tr>
<tr>
<td>6-Palmitate</td>
<td>B 0 0 0</td>
</tr>
<tr>
<td>Canthaxanthin</td>
<td>B 0 0 0</td>
</tr>
<tr>
<td>Trolox C</td>
<td>B 0 0 0</td>
</tr>
<tr>
<td>Acetylcycteine</td>
<td>B 0 0 0</td>
</tr>
<tr>
<td>Coenzyme Q_{0}</td>
<td>B 0 0 0</td>
</tr>
<tr>
<td>(+)-Catechin</td>
<td>B 0 0 0</td>
</tr>
</tbody>
</table>

In animal tissue studies, heme proteins are the major chromophoric compounds in the visible region of the spectrum. Heme compounds change from one compound to another upon oxidation. HPSAP transmission spectra of tissue slices or homogenates reveal the spectral absorption of all the elements in the tissue and thus have high turbidity. Turbidity is a major problem in the use of the method. On top of the spectrum of the turbidity are the spectra of the heme compounds. To deconvolute the spectra, the literature standard spectra of hemoglobin and its derivatives—deoxyhemoglobin, oxyhemoglobin, methemoglobin, and ferrhemoglobin—are used. We also incorporate the standard spectra of the hemochromes and hemichromes that can be formed as well as minor components, such as the cytochromes of mitochondria and microsomes. Because all these pigments are present in tissue, for example heart tissue, it is a major problem to deconvolute the spectra of the combined absorptions of all pigments into the spectral absorption of each component and thus determine the quantity of each oxidized heme pigment. We have been able to do this. The spectrum of heart homogenate shows in the visible region the major peak of oxyhemoglobin and in the near-ultraviolet region the corresponding γ peak of the heme protein (Figure 3). After the homogenate is allowed to oxidize, the spectrum indicates principally methemoglobin with the corresponding γ peak in the near-ultraviolet region. The spectrum also incorporates the turbidity absorption shown under the spectrum of the major heme proteins which must be subtracted from the absorption of the heme proteins. The study of oxidized heme proteins is useful in experiments of tissues and homogenates being oxidized. Elevated levels of heme protein oxidation correlate with increases in TBARS and enzyme inactivation, another method of measuring oxidative damage.

In another experiment (7), antioxidant nutrients are added to the basal diet (B) to provide maximum protection (Table 2). No antioxidants are added to the control basal diet (B). The second diet (E + Se) contains enough vitamin E and selenium to offer sufficient protection against normal conditions. For maximum defense against oxidative conditions, the third diet (ALL) contains multiantioxidant nutrients including β-carotene, canthaxanthin, coenzyme Q_{10} and coenzyme Q_{0} to provide the antioxygenic activity of coenzyme Q, trolox C, a more water-soluble model of vitamin E, ascorbyl palmitate as an ascorbic acid antioxygenic agent since the rat makes its own ascorbic acid and (+)-catechin, a polyphenol such as those present in fruits and vegetables. A summary of the results is shown in
Figure 4. Oxidized heme proteins develop as a function of time with the highest amount forming in the liver of animals on the basal diet. Some protection is given by the vitamin E and selenium diet. Further protection against oxidative damage to the liver is provided by the diet containing multiantioxygenic agents. From a practical standpoint, this is probably maximum protection of tissue. The amounts of antioxidant nutrients added to the diets are within the range humans can receive from their food supply or supplementary additions to their diets.

In conclusion, focusing on experimental results from our laboratory, we find that animal tissue experiments are very valuable. Antioxidant protection by multiantioxygenic agents can be quantitated. The evidence indicates that multiantioxygenic nutrients can achieve maximum protection. Considering the biochemistry of β-carotene and our studies of its activity, apparently, the popular β-carotene is a weak antioxidant.

References

Trans Fatty Acids in the Food Supply: A Comprehensive Report Covering 60 Years of Research, 2nd Edition©1995 by Dr. Mary G. Enig

There is little question that trans fatty acids are the new pariah of the Nineties. Don't you owe it to yourself to learn why? Don't play "catch up" with your competitors in the international marketing for edible fats and oils. The time to learn is now!

Authored by Dr. Mary G. Enig, an internationally recognized trans fatty acid expert, Director of the Nutritional Sciences Division of Enig Associates, Inc., and former Faculty Research Associate with the Lipid Research Group of the University of Maryland, this second edition - over 200 pages with more than 55 tables and figures -- includes the following topics: health effects reported in animals and humans; physical and chemical properties; the hydrogenation process; analysis by, e.g., IR, GLC; natural and man-made sources; levels in the diet based on consumption surveys and availability; who's doing the research; labeling; dietary guidelines; and database issues. This report will be of great interest to those involved in manufacturing, marketing, sales, and distribution of edible fats and oils, and nutritional/medical scientists and clinicians.

For a copy of Trans Fatty Acids in the Food Supply, 2nd ed. (available Summer 1995), please send US$145.00 (shipping and handling included; additional copies at $100.00 per copy) by check payable to Enig Associates, Inc.

Write to: International Marketing Division, Enig Associates, Inc., Suite 500, 11120 New Hampshire Ave., Silver Spring, MD 20904-2633 U.S.A., or call (301)593-4471 or fax us at (301)593-9793 for quickest response.

Internet Users: to receive an order form, go to the Trans Fat Info Web at http://www.enig.com/trans.html or email to: sustrop@aol.com

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HEALTH & NUTRITION

A symposium on nutrition, atherosclerosis

A symposium on Nutrition and Atherosclerosis was held at the Loews Le Concorde Hotel in Québec City on Oct. 6–8, 1994, as a satellite of the Xth International Symposium on Atherosclerosis held in Montréal the following week. The host society for these events was the Canadian Atherosclerosis Society.

The satellite symposium opened with an introductory session on the evening of Thursday, Oct. 6. The delegates were welcomed by the honorary chairman of the 10th International Symposium, M. Daria Haust, Department of Pathology, The University of Western Ontario, London, Ontario. There followed presentations by Paul Nestel, Chief, CSIRO Division of Human Nutrition, Adelaide, Australia, and Henry McGill Jr., Department of Physiology and Medicine, Southwest Foundation for Biomedical Research, San Antonio, Texas.

Dr. Nestel emphasized the rapidity and magnitude of changes in coronary heart disease (CHD) that have occurred during the past 25 years in different parts of the world. Whereas the prevalence has declined markedly in countries such as Australia and the United States, it has risen dramatically in Eastern European countries. Increases also are being observed in some Asian and Pacific countries. These data emphasize the reversibility of CHD by factors such as diet.

In dietary recommendations regarding CHD, reduction of saturated fat has been stressed most, but there is strong evidence from epidemiological data that other dietary components, such as fish and plant foods, may exert protective effects. In particular, there is increasing evidence that dietary antioxidants play an important role in preventing CHD. Dietary modification also can play an important role in reducing the impact on CHD of risk factors such as obesity and hyper-tension.

Dr. McGill reviewed the literature on individual variations in lipemic responses to dietary fat and cholesterol in both humans and experimental animals. These variations appear to be due to genetic control of metabolic variables such as cholesterol absorption, bile acid secretion and apo B production rate. The mechanism may differ between species and also among individuals.

The Friday morning session was devoted to effects of dietary macronutrients of various aspects of cholesterol metabolism. Emile Levy, Centre de Recherche, Hôpital Ste-Justine and Department of Nutrition, Université de Montréal, Montréal, Quebec, reported results showing that essential fatty acid deficiency in rats decreased the rate of bile acid secretion as well as the activity of HMG-CoA reductase and acyl CoA:cholesterol acyltransferase. K.C. Hayes, Foster Biomedical Research Laboratory, Brandeis University, Waltham, Massachusetts, reviewed evidence that saturated fatty acids differ in their effects on serum cholesterol and lipoprotein levels. In particular, fats rich in lauric (12:0) and myristic (14:0) acids were found to be more hypercholes- terolemic than those rich in palmitic acid (16:0) or stearic acid (18:0). The effect of palmitic acid appears to depend on other factors such as the level of cholesterol in the diet. The position of fatty acids on the triglyceride molecule also may be a factor. Martijn Katan, Department of Human Nutrition, Agricultural University, Wageningen, The Netherlands, discussed the effects of dietary trans fatty acids, which have been shown in his work to raise the levels of LDL (low-density lipoprotein) and Lp(a) [lipoprotein (a)], which are athero-genic, and to lower the level of HDL (high-density lipoprotein), which is antiatherogenic. It was suggested that these effects may be mediated through cholesterol ester transfer protein.

Christian Drevon, Institute for Nutrition Research, University of Oslo, Oslo, Norway, reviewed nutritional aspects of n-3 fatty acids, found mainly in fatty fish and fish oils, which reduce plasma triglycerides, probably by decreasing hepatic secretion of VLDL (very low density lipoprotein) and by increasing catabolism of chylomicrons. These highly unsaturated fatty acids are susceptible to peroxidation, but such effects may be counteracted by dietary antioxidants. The role of dietary fish protein in regulation of plasma lipids was discussed by Hélène Jacques, Department de Nutrition Humaine et de Consommation, Laval Université, Quebec, Quebec. Studies on rabbits and on pre- and postmenopausal women indicated that substitution of lean white fish for protein from other animal sources did not improve the plasma lipid profile. The relationship between dietary fiber and coronary heart disease was the subject of the presentation by James W. Anderson, Metabolic Research Group, VA Medical Center and University of Kentucky, Lexington, Kentucky. Animal studies and clinical trials have both

This article was prepared for INFORM by Kenneth K. Carroll of the Department of Biochemistry and Director of the Center for Human Nutrition, The University of Western Ontario, London, Ontario N6A 5C1, Canada, assisted by Yves Deshaies, Pierre Julien, Henry Ginsberg and Scott Grundy. Full presentations will be printed in the Canadian Journal of Cardiology.
provided evidence of hypocholesterolemic effects of viscous, soluble fibers, such as psyllium, oat gum, guar gum and pectin. Increased fiber intake may also decrease blood pressure, assist in weight management after blood clotting and increase insulin sensitivity.

The Friday afternoon session was devoted to presentations on nutrient-genetic interactions. The intent of this session was to examine the variability in response to diets due to lipoprotein lipase (LPL) regulation and to polymorphism in apolipoprotein genes. Thomas Olivecrona, Department of Medical Biochemistry and Biophysics, Umeå University in Umeå, Sweden, reviewed the various steps involved in the modulation of LPL activity and pointed out the tissue specificity of LPL regulation in various physiological states. It was concluded that LPL is a multifunctional protein which acts not only as a lipolytic enzyme, but also as a ligand involved in the binding of lipoprotein to cell surfaces and receptors. Mavis Abbey, CSIRO Division of Human Nutrition, Adelaide, Australia, addressed the controversial issue of apolipoprotein polymorphisms in the response to dietary changes. Apo B polymorphisms have been reported to be associated with coronary disease and hyperlipidemia, but present studies indicate that the apo B EcoR1 RFLP phenotype is not associated with response to diets. It was indicated that response to dietary changes is most likely polygenic, and analyses of multiple haplotypes would be required to strengthen the association between genetic factors and individual response to dietary lipids. In a similar vein, Gustav Schonfeld, Washington University School of Medicine, St. Louis, Missouri, studied polymorphic markers of the apo B gene scattered along the length of the gene. The largest effects on fasting lipid levels were demonstrated by gene mutations affecting the apo B structure, as seen in familial hypobetalipoproteinemia where a number of truncated apo B proteins have been described. It was pointed out that the kinetics of apo B lipoproteins depend on the lengths of the various apo B truncations. Finally, the symposium participants heard from Matti J. Tikkanen, University of Helsinki, Helsinki, Finland, that the role of apo E gene variations in the modulation of the response to dietary cholesterol remains controversial. Lipid metabolism associated with polymorphisms in the apo E allele was initially reviewed and showed association between the E4 genotype and the response to plasma lipids. Further dietary studies are required to elucidate the potential effect of apo E genetic variation on the regulation of dietary response. In summary, even though it is evident that genetic factors have determinant effects on the dietary response, the enormous complexity of nutrient metabolism interactions with genetic variants of plasma apolipoprotein is readily evident.

The third speaker in this session was Mario Mancini, Institute of Internal Medicine and Metabolic Diseases, Federico II University, Naples, Italy, whose topic was the Mediterranean diet in relation to cardiovascular disease. A comparison of diets of healthy young people in Naples and in Bristol, England, showed that the Naples group consumed more tomatoes and tomato juice, more monounsaturated fat, with higher levels of vitamin E and β-carotene, Plasma lipid peroxidation was significantly lower in the Naples group, which may contribute to lower risk of cardiovascular disease.

The Saturday morning session featured three presentations on selected topics, followed by a workshop on The Optimal Diet for Reducing Risk of Atherosclerosis. The first speaker in the selected topics session was I. Jialal, Center for Human Nutrition, University of Texas Southwestern Medical Center, Dallas, Texas. His presentation focused on effects of vitamin E, vitamin C and β-carotene on LDL oxidation and atherosclerosis. Natural antioxidants are preferable to antioxidant drugs, which may have undesirable side effects. The potential of antioxidants, especially vitamin E, for preventing the development of atherosclerosis is promising, but clinical trials are needed to prove that these antioxidants are beneficial. Barry Lewis, St. Thomas' Hospital, University of London, London, United Kingdom, reported results of the St. Thomas Atherosclerosis Regression Study (STARS) on men with symptomatic coronary heart disease who were given a lipid-lowering diet, with or without cholesteryamine, or managed with usual care. The results showed that the treatment was beneficial, as measured by quantitative coronary angiography and measurement of blood lipids. Dietary saturated fatty acids from C14 to C18 were all positively associated with progression of atherosclerosis.

The workshop on the optimal diet began with brief presentations by Roger Illingworth, Department of Medicine, Oregon Health Sciences University, Portland, Oregon; Donald McNamara, Department of Nutritional
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Sciences, University of Arizona, Tucson, Arizona; Ernst Schaefer, USDA Human Nutrition Research Center on Aging, Tufts University, Boston, Massachusetts; David Jenkins, Department of Nutritional Sciences, University of Toronto, Toronto, Ontario; and Bernard Wolfe, Department of Medicine, University Hospital, London, Ontario. This was followed by a general discussion moderated by Henry Ginsberg, Department of Medicine, Columbia University College of Physicians and Surgeons, New York, New York, and Scott Grundy, Center for Human Nutrition, University of Texas Southwestern Medical Center, Dallas, Texas.

Dr. Iltingworth presented data for subjects on high, medium and low cholesterol diets to show that dietary cholesterol increases LDL cholesterol and suppresses endogenous cholesterol synthesis. The effect on LDL cholesterol was greatest in the subjects with pre-existing hypercholesterolemia. Dr. McNamara concluded from an analysis of 128 cholesterol feeding studies involving 2,750 subjects conducted over a period of 30 years that, on average, a change in cholesterol intake of 100 mg/day alters plasma total cholesterol by only 0.07 mmol/L (2.5 mg/dL). The response was independent of amount of dietary fat and baseline plasma cholesterol level and showed considerable individual variability. Dr. Schaefer reviewed current recommendations on dietary fat for risk reduction of coronary heart disease in the general population. It is still not clear whether 30% of energy is the optimal level for total fat. Although Mediterranean diets are relatively high in fat, principally olive oil, age-adjusted mortality rates are lower in southern compared to northern European countries or the United States. However, both fat intake and mortality are lower in Japan. Fat restriction promotes weight reduction as well as lowering LDL, so greater restriction of dietary fat can be recommended for overweight individuals. Dr. Jenkins stressed the desirability of increased consumption of plant foods, including green leafy vegetables, nuts and seed, and dried legumes. Such foods can improve the overall nutritional quality of the diet by providing specific ingredients, such as soluble viscous fibers, vegetable proteins, antioxidants such as vitamin E and flavonoids, as well as polyunsaturated fatty acids. This represents a return to a more primitive diet on the evolutionary scale. Dr. Wolfe presented evidence that substituting protein for carbohydrate in the diets of moderately hypercholesterolemic or familial hypercholesterolemic subjects significantly lowered total and LDL-cholesterol and triglycerides, while raising HDL-cholesterol. He suggested that this may be of practical value in the treatment of patients with common metabolic disorders underlying coronary heart disease.

In the discussion following these presentations, Dr. Grundy noted that the small decrease in serum cholesterol that could be achieved by reducing dietary cholesterol intake is not trivial in terms of reduced risk for atherosclerotic cardiovascular disease. Dr. Hayes commented that the interaction of dietary cholesterol with dietary fat was quite significant, as least in animals. He thought this interaction made individuals consuming low-cholesterol diets less responsive to saturated fatty acids. Dr. Nestle noted that HDL-cholesterol increases with increased dietary cholesterol intake, particularly in young females. In the latter, both LDL and HDL increase. Dr. Ginsberg stated that his group had observed similar responses in young females. HDL concentrations do not rise significantly in males. Dr. Carroll reminded the audience that the Canadian dietary guidelines make no specific recommendations regarding dietary cholesterol; the approach in Canada is to recommend a diet that will reduce all chronic disease, and this means focussing on dietary fat and caloric intake. Dr. McNamara concluded this discussion by suggesting that the primary focus should be on saturated fats.

Drs. Jenkins and Ginsberg initiated a discussion regarding the significance of HDL cholesterol, particularly in the context of diet-induced reductions. Both felt that too much attention was focussed on lower HDL-cholesterol levels seen on very low-fat diets. Dr. Grundy remarked that he continues to be concerned about the pattern of low HDL and elevated triglyceride levels, even in the context of a low-fat diet. Dr. Schaefer returned to the theme of low-fat diets and weight loss. He felt that weight reduction would result in lower LDL and higher HDL cholesterol levels, even when the diet was high in carbohydrates. Dr. Ginsberg mentioned that a multicenter study comparing diets in which saturated fat has been replaced by either carbohydrate or monounsaturated fats is underway in the United States.

The meeting ended with discussions centering on weight loss and obesity. It was agreed that artificial fat substitutes might increase obesity even more, and that mean body weight in the United States had increased during a decade when artificial sweeteners and fat substitutes had filled the grocery stores. Obesity and caloric excess were felt to be the greatest problems facing both Western nations and nations emerging from subsistence. Although the many problems we face in designing the optimum diet, and in gaining acceptance of such a diet by society, seem significant, the potential benefit to be gained by facing these problems and solving them is great.

Members of the meeting planning committee were K.K. Carroll, chairperson; Paul Nestle, cochairperson; Murray Huff, Hélène Jacques, Pierre Julien, Émile Levy and Bernard Wolfe.
Because it appears that heart disease already begins in youth, what people eat can affect their health many years later, Margo Denke reminded attendees at the Edible Applications Division luncheon held Tuesday, May 9. Approximately 40 persons were present.

Denke, assistant professor of internal medicine and a researcher at the Center for Human Nutrition, University of Texas Southwestern Medical Center at Dallas, added that although many risk factors—such as smoking, age, high blood pressure and serum cholesterol—have been identified for coronary heart disease (CHD), there are other dietary effects that are unknown. Some epidemiological studies have indicated a slight correlation between total fat and higher risk. However, an even greater correlation has been found linking saturated fat intake to increased risk.

"In fact, saturated fatty acids are the most potent dietary factor that affects LDL (low-density lipoprotein) cholesterol levels," Denke said. "The more saturates you eat, the higher your LDLs."

She added, "Diet changes seem to have more effect on persons with mild to moderate cholesterol levels and thus could be used to reduce the number of individuals at risk for heart disease."

Other developments in the area of lipids and health include work showing LDL oxidation as a factor in atherosclerosis and the potential for antioxidant vitamins to reduce oxidative susceptibility. Polyunsaturated fatty acids appear to be more susceptible to oxidation, while monounsaturated fatty acids, which don't raise blood cholesterol, are less susceptible.

Although there is no conclusive epidemiological proof yet that vitamins lower risk, Denke told those taking antioxidant vitamins, "Good luck. Maybe it will work, and maybe it won't."

She added, "My guess is the answer is a little more complicated than that. My theory is that saturated fats are more important than antioxidant vitamins."

Meanwhile, a diet-and-cancer link is even more difficult to ascertain than that for CHD. One challenge is that specific types of cancers are much less common than CHD, and thus it is difficult to study a population sample.

U.S. 1989 data indicated 34% of deaths (734,000 persons) were from CHD, and 23% (496,000) were from cancer. Of these deaths, 6% were attributed to lung cancer (137,000 persons), 3% to colon/rectal cancer (57,000 persons), 2% to breast cancer (43,000 persons) and 1% to prostate cancer (30,000 persons).

"Also, risk factors and disease markers have yet to be identified for many cancers," Denke said. "It's a difficult nut to crack."

"We do know that increased fruit and vegetable consumption is associated with reduced cancer rates, but whether this is due to the vitamins or something else or because the fruits or vegetables replace other foods in the diet is unknown," Denke said. The antioxidant hypothesis, she said, is an exciting area, "but we don't yet have the answers."

Luncheon attendees also were given a look at biotechnology and its application to food, fats and oils. Willie Loh, director of research and development for Cargill Foods, outlined both technology and production aspects related to genetically modified oils.

Currently, the only modified oils available on the market have been produced using conventional mutation procedures, Loh said. However, work is under way by various com-
Kritchevsky provides musical commentary

David Kritchevsky

Annual meeting participants attending the Health and Nutrition Division noon luncheon on Wednesday, May 10, were entertained with a special performance by David Kritchevsky of the Wistar Institute.

Kritchevsky, a member of AOCS for 36 years and a pioneer in the area of atherosclerosis and lipids, gave a brief talk on "Fifty Years of Lipid Research—So What Have We Learned?" followed by a performance at the piano. Familiar tunes such as the Battle Hymn of the Republic, Jingle Bells and from the musicals Oklahoma! and Fiddler on the Roof were given a new twist as Kritchevsky sang lipid-related lyrics he has penned. In his lyrics, Kritchevsky demonstrated how he teaches biochemistry, providing a musical commentary on research grants ("If I had a big grant") and poked fun at lipid research ("We feed them, then we bleed them").

In his talk, Kritchevsky urged those in the health and nutrition field to not take themselves too seriously. "You have to keep an open mind, because there are very few things that are absolute."

Noting the complexity of nutritional issues, Kritchevsky cited various areas that have been studied over the years: first fat, then saturated and unsaturated fats, the polyunsaturated-to-saturated fat ratio, cholesterol, high-density lipoproteins and low-density lipoproteins (LDL) and now the size of LDL. "All of the advances march on the feet of methodology," he said, pointing to the advances in analytical tools available to researchers.

However, U.S. consumers may be too preoccupied over health and nutrition, he said, pointing out, "We're a nation of the worried well." Americans, he said, "think death is an option."

Also, he pointed out, the trouble with diet recall

(continued from page 787)

panies to produce "strategic products" using gene cloning, gene expression and plant transformation.

"There will come a time when strategic products will take over," Loh predicted.

The advantage currently of using conventional mutation is that the resulting products do not require regulatory review. However, eventually, companies probably will use both conventional mutation and gene cloning and transformation, he said. "Right now, patents are an important issue. The dilemma is will we be able to use the traits developed?"

Identity preservation is an important consideration in producing and safeguarding genetically modified oils, Loh said. He presented data listing the main players and partners—Dupont/Cargill, Mycogen/SVO, Pioneer/Kraft, Calgene/Hunt Wesson, the University of Manitoba/CanAm era and Western Grower Seed Company/Bunge. Modified oils either commercially available or under development include: low-linolenic canola; high-oleic canola; low-linolenic, high-oleic canola; high-oleic sunflower; low-linolenic soy; and high-oleic soy oil.

Current division officers are Frank Orthoefer of Riceland Foods, chairperson; Neil R. Widlak of Campbell Soup, vice chairperson; and Anthony V. Petricca of SVO Specialty Products Inc., secretary. Jerry Hasenhuettl of Kraft General Foods was appointed to a committee to nominate officer candidates.

In other division business, Widlak announced that he will serve as chairperson for an AOCS world conference on structured lipids that is being planned for 1997. Hershey, Pennsylvania, is being considered as a possible site for the conference.

The division had 33 paid members at the time of its luncheon meeting in May and $1,300 in its treasury. It helped organize nine technical sessions at this year's AOCS annual meeting.

Red wine is topic for protein dinner

If you're planning to drink a toast to good health, make it red, red wine. Such was the recommendation of Roy Williams of the oenology (wine science) program in the department of chemistry at Old Dominion University in Virginia. He presented his entertaining and informative message to approximately 45 members of the Protein and Co-Products Division at their annual dinner meeting held in conjunction with the AOCS 86th Annual Meeting and Expo in San Antonio, Texas.

Cartoons by a professional illustrator accompanied Williams' review of the international aspects and history of wine production and consumption. He joshed about Australian Kangaroo red wine, Texas Chateau Infidel Zinfandel, showed a
studies is that people don’t accurately recall what they ate and when questioned, “compensate on the side of less, not more.”

The approximately 90 luncheon attendees gave Kritchevsky a standing ovation after his talk and performance at the piano.

Mark Bieber of Best Foods, division chairperson, reported that the section has 414 members. Students may become members of the division without charge.

Bieber told the approximately 90 persons at the luncheon they had until the end of May to cast ballots to elect division board members. Richard Cantrill and Margaret Craig-Schmidt were listed as candidates for secretary/treasurer, while Susan Czarnecki, Mary Enig, Robert Nicolosi and Ronald Yarger were candidates for two member-at-large positions. Bieber will continue as chairperson, with Vic Huang of Ross Products Division, Abbott Labs, as vice chairperson. Mel Mathias of the U.S. Department of Agriculture will continue as a board member-at-large until 1997.

At this year’s annual meeting, the division helped organize ten sessions, including two symposia—one on trans fatty acids and a second on gamma-linolenic acids.

In a roundtable discussion Monday afternoon, May 8, division representatives worked on session planning for the next several years. Plans for 1996 include possible sessions on conjugated dienes, patient-adapted modified fats, nutritional regulation of gene expression, lipids and signal transduction, animal and dairy lipids, general health and nutrition and biochemistry topics, fat nutrition policy, and a joint session with the Oxidation Division on antioxidants, nutrients and fatty acid metabolism.

menage of ethnic groups washing grape vines (“Ethnic Cleansing,” he said), and termed the current debate about wine trade in Europe as the “European Community Festival of Sour Grapes.”

Then he zoomed in on France, long revered for its fine wine and recently in the news because of “The French Paradox.”

The French Paradox first appeared in the literature reporting a World Health Organization (WHO) survey long ago covering the age, diet and other demographic factors of people around the world and examining the countries’ rates of heart disease, Williams said. Data indicated that the French, who consume saturated fat at high levels, didn’t have the rate of heart disease expected. The French are known not only for making and exporting wine. They drink it—copiously.

However, the WHO report was largely ignored until 1992, when scientists postulated that the French wine consumption might have a role in deterring heart disease, Williams said. Although fine wine often is linked with romance and young love, Williams’ task was to study how wine literally could be good for the heart.

His work has identified the key compound as polyphenolics in the seeds and skin of varietal grapes used to make wine. Polyphenolic compounds are antioxidants, Williams said.

Red wines have more polyphenolics than white wine, according to Williams, because in making red wine the grapes are crushed with the seeds and skins and then fermented. In making white wine, the grapes are pressed into juice and the seeds and skins are filtered out before the juice is fermented. Thus, while white wine may have from 10 to 35 mg/L, red will have from 200–300 mg/L, Williams said. Two glasses of red wine may deliver 120 or so mg of polyphenolics to the imbiber’s blood.

People have known about the health benefits of polyphenolics for hundreds of years, according to Williams. French sailors in the 1600s took a potion concocted from pine
bark and needles to ward off scurvy. The bark and needles were high in polyphenolics. Later, scurvy preventives were made from grapeseed extract.

In Japan, a drink brewed from knotweed has been used as a potent antioxidant because knotweed is high in polyphenolics, Williams said.

Today's anti-aging skin creams contain polyphenolics, and the French are conducting a variety of hospital-based clinical trials on polyphenolics. In 1993 there were some papers on polyphenolics published in the United States, "but America hasn't kept up with work being done in Europe," Williams said.

"You can buy pills that are the equivalent of two glasses of wine," Williams said. However, if you want to get your polyphenolics from pills, go to Canada to buy them; they're illegal to sell in the United States. "But I'm a firm believer in getting polyphenolics from a good glass of wine," Williams said. Polyphenolics also occur in onions, garlic and tea.

Williams has conducted research into the effects of polyphenolics on cell cultures and said "it looked like the cells are being rejuvenated. They looked better than the untreated control cells." He has also induced thrombus in canine aorta to simulate heart disease and then injected polyphenolic compounds. The diseased condition was eliminated, he reported.

He has also been experimenting with fermenting white wine (Chardonnay) on grape seeds to increase the wine's polyphenolic levels while maintaining the wine's traditional flavor, color and character. He also has data showing that polyphenolic levels increase as alcohol levels increase because the alcohol is a solvent that extracts the polyphenolics from the seeds and skins used in the fermentation process.

Also at the dinner, the Archer Daniels Midland Award for chemistry-nutrition was accepted by Hiroshi Harada for a team-written paper, "Determination of Soybean Antigenicity and Reduction by Twin-screw Extrusion". Co-authors were Akio Ohishi, Kazunori Watanabe, Masumi Urushibata, Kimiko Utsuno, Kazuya Ikuta and Karou Sugimoto.

New officers were elected: Fred Shih, chairperson; Edith Conkerton, vice chairperson; and Chunyang Wang, secretary/treasurer.

The Protein and Co-Products Division reported membership of 103. Treasurer Edith Conkerton said that the division treasury had a balance of $5,995 and that sales of sponsored T-shirts were expected to add to that total.

President Fereidoon Shahidi reported that the AOCS national effort to increase membership will focus on universities with programs whose participants might become interested in AOCS membership and noted the offer of free membership for students in their first year of membership.

Phosphatidylcholine's role in cancer discussed

Forty-five persons attended the Phospholipid Division luncheon on Monday, May 8 to hear Steve Zeisel review his research on how phosphatidylcholine helps cells ward off cancer.

From his 20-year perspective of research, Zeisel said that scientific thought on phospholipids has changed dramatically from viewing them as merely structural components supporting proteins to recognizing their functions in cell signaling.

"They are key players in how signals are sent from outside of a cell to the inside of the cell," Zeisel said.

Some of those signals trigger the initiation and growth of cancers. The most recent development in the field has been the study of how cells die—it is now known that cells contain death programs called apoptosis, a topic that now has generated 2,500 published papers, he said.

Zeisel said that cells apoptosis is "turned on" at some point during normal cell life and the cell destroys itself, or "commits suicide," he said. This idea was presented in 1971 and confirmed by research that showed that a certain species of caterpillar has to kill off 131 cells, via apoptosis, in order to become a butterfly, according to Zeisel. "But the concept was ignored by mainstream scientists."

"Cells have a number of divisions to make before they turn on apoptosis," Zeisel said.

He presented a brief summary of the biochemistry of choline and its derivatives such as phosphatidylcholine and protein kinase C.

Some of Zeisel's research has shown that choline availability during pregnancy and lactation is important to the development of memory areas in the brain of offspring. At the luncheon he discussed development of liver cancer in rats deprived of choline. "Worldwide, liver cancer is the leading form of cancers," he said.

He also said, "It has also been shown that choline-deficient animals are more sensitive to environmental and chemical carcinogens " than are animals with normal levels of choline.

It is possible to culture liver cells from choline-deficient animals and create choline-deficient cells for research. This research has shown that choline is essential for cell survival, he said, adding that cells deprived of choline die by apoptosis.

He reviewed how genes and a series of known molecules interact to turn on the apoptosis signal, and how

(continued on page 792)
the lack of a cell death signal allows cancer cells to develop.

He summarized research involving reducing the amount of choline provided to cell cultures over several months. The cells that survived were cells "that wouldn't die for lack of choline," he said. "They may have lost the ability to commit suicide and they became cancer cells that formed tumors when injected into mice." Thus, choline is an important factor in cell regulation.

"This is a model of a nutrient deficiency that leads to cancer," he said.

### AICR plans meeting on phytochemicals


The program will include separate sessions on isothiocyanates, polyphenols, flavonoids, monoterpenes and organosulfides, as well as a session on phytochemicals in the U.S. diet and their role in cancer prevention. This last session will feature two panels: one on educational and practical aspects of plants in human nutrition, and one on phytochemicals and the food industry. There also will be a poster session.


### Brain Development: Relationship to Dietary Lipid and Lipid Metabolism

J. Jumpsen and M.T. Clandinin

The development of the brain is an important consideration for students of nutrition, lipid scientists, and other health-care professionals concerned with the growth of premature infants and for researchers involved in developing new infant formulas and infant foods. With an increase in the number of surviving premature infants and a growing interest in developing the "gold standard" for infant formulas, bringing together information regarding brain development and lipids may be beneficial and useful for many. The requirement and timely availability of the correct nutrients are critical because brain development is such a precise, complex, and one-time-only event. Limiting this book to only a concise discussion of lipids was not easy. It was accomplished by examining the different lipids and their roles in the brain, an organ highly concentrated in lipids. This book's objectives are to both provide a focused overview (morphological, biochemical, and functional) of brain development and to exemplify the role of lipids in the important developmental events and the concepts that are potentially altered by physiological changes in brain lipid composition.

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**L.L.M. van Deenen, noted researcher, dies**

Dr. Laurens L.M. van Deenen, recipient of the 1981 AOCS Award in Lipid Chemistry, has died.

A recent newsletter from the International Conferences on the Biochemistry of Lipids said Dr. van Deenen died Sept. 4, 1994. He was internationally known for his research on membranes and was a pioneer in research on the role of phospholipids in membrane permeability.

He was a long-time faculty member at the University of Utrecht, where he had received his doctorate in 1957. Dr. van Deenen had served on the editorial board for several scholarly journals. Among his other awards were the Heinrich Wieland Award in 1971, the Dr. H.P. Heineken Prize in 1976, and the Diplome d'Honneur of the Federation of European Biochemical Societies in 1979.

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