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HOMOGENIZED MILK AND CORONARY ARTERY DISEASE

THEORY, NOT FACT

There has been widespread coverage in news media recently of the claim that the drinking of homogenized milk is a cause of excessive mortality from coronary artery disease in the United States. That this relationship might exist has been proposed by Oster (1) who postulates the existence of a group of diseases caused by the depletion of plasmalogen in cell membranes. He includes arteriosclerosis, myocardial infarction and angina pectoris as representative disorders with this possible origin.

Plasmalogens are a type of phospholipid usually found as a component of cell membranes of muscle and of the myelin sheath of nerve fibers. The major plasmalogen of myocardium is a choline phosphatide, containing an unsaturated fatty acid in a typical ester linkage and a second fatty acid as a vinyl ether (2), thereby differing from the more usual phospholipid exemplified by lecithin. Fatty aldehydes or plasmals can be released from this second linkage by action of an enzyme, vinyl etherase, on plasmalogens. This plasmal reaction takes place under highly acid in vitro conditions (pH 1) and the use of fuchsin and sulfurous acid permits staining of plasmalogens in tissue sections. Oster, utilizing this histochemical procedure on sections of myocardium from one patient dying acutely of coronary artery disease, was unable to demonstrate plasmalogens (3) and later reported absence of plasmalogens in fatty streaks of the aorta of a young drowning victim (4). From these observations deriving from the two autopsies, he concludes that plasmalogens

disappear from myocardium promptly after infarction and are not present in the earliest lesions of atherosclerosis. Other studies which have determined the quantity of aldehydogenic plasmalogens after extraction from several hundred human aortas, report a decrease with age and with the severity of the atherosclerotic lesion (5,6). These reports do not include observations on the plasmalogen content of the myocardium.

Bovine xanthine oxidase is an enzyme found in milk that can oxidize plasmal aldehydes in vitro (7). Thus, according to Oster, palmitaldehyde formed from the tissue breakdown of plasmalogens could be converted to palmitic acid producing damage to the myocardium by depletion of necessary membrane components. Since normal myocardium contains little or no xanthine oxidase (in man the greatest activity of this enzyme is found in liver and intestinal mucosa), Oster reasoned that perhaps the enzyme might play a role, if it came from an extrinsic source, (such as in milk consumed as food), was absorbed and deposited in active form in the myocardium, and was active under in vivo conditions after the plasmal reaction had taken place. In an effort to demonstrate a possible role for xanthine oxidase, activity of the enzyme was determined in the aortas of four patients aged 54 to 75 years with atherosclerotic disease and in the myocardium of two of these (8). Similar studies were done on the aorta of a 37 year old man without obvious atherosclerosis, who died of trauma. Oster reports that more than minimal xanthine oxidase activity was found only in the aorta and myocardium when there was gross evidence of atherosclerotic change. However, the analyses are few in number and descriptive information is limited. No effort was

made to characterize the enzyme in terms of bovine or human origin.

Another line of argument is offered to support the role of bovine xanthine oxidase. Oster sees in the homogenization process the likelihood of more ready absorption of the enzyme. Since xanthine oxidase is bound to lipid globules in milk and homogenization increases the proportion of such globules of small size (1 micron or less), he believes that there is greater likelihood of intestinal absorption of the intact enzyme. He adds that death rates from coronary artery disease have increased in the U. S. since introduction of the homogenization process in the 1930's, and that populations in other countries who characteristically consume boiled or curdled milk do not have high mortality rates. These arguments fail to take cognizance of the fact that heart disease mortality rates began their increase well before there was widespread use of homogenized milk in this country and have been decreasing in the last decade.

Thus the hypothesis as evolved states that ingestion of homogenized cow's milk leads to absorption of active xanthine oxidase in the gut and deposition of this enzyme in the myocardium. This in turn is associated with breakdown of membrane plasmalogens and depletion of tissue fatty aldehydes causing myocardial damage. There is no solid evidence to support any step in this postulated sequence of events.

Although carefully designed experiments have brought conclusive evidence that protein macro-molecules may be absorbed in the intestine of experimental animals in biologically and immunologically active forms and quantities (9), these experiments have involved

proteins with molecular weights of 80,000 or less. In contrast, the bovine xanthine oxidase molecule has a weight approximating 300,000 and this size would seem to preclude absorption in intact form. Furthermore, at the pH of the stomach, xanthine oxidase would be irreversibly denatured and its active groups cleaved (10). Thus, crucial to the hypothesis advanced by Oster, is the need to demonstrate unequivocally, in the face of its unlikelihood, that xanthine oxidase in homogenized cow's milk is absorbed intact and retains biologic activity in human tissues. An effort has been made to bring such evidence (11). Unfortunately, it is not convincing and raises many questions concerning its scientific validity. The procedure used was a test for antibodies in human sera using a partially purified bovine xanthine oxidase as antigen in a tanned erythrocyte system. The sensitivity and specificity of the reaction was not assessed and a crude measure of antibody potency was utilized. Blood sera were obtained from two groups of patients seen by an internist. One group of 41 patients described as a control group was stated to have no clinical evidence of atherosclerotic disease. The second group of 34 patients had a variety of manifestations ascribed to atherosclerosis, i.e. angina pectoris, myocardial infarction, claudication, peripheral vascular disease, cardiac arrhythmias and chronic brain syndrome. The two groups are not comparable in age or sex distribution and the control group includes patients with hypertension and diabetes mellitus, who would be likely to have some atherosclerotic involvement. The authors claim to have demonstrated specific antibody to bovine xanthine oxidase in the sera of both groups of patients and with somewhat higher titers in the patients with

atherosclerotic disease. These results, however, do not insure that the bovine enzyme is absorbed, is biologically active, or that it is in fact deposited in the target tissues (arterial wall and/or myocardium). Even if it were present, there is no evidence that this enzyme could contribute, under in vivo conditions, to the breakdown of membrane plasmalogens. Under any circumstance, the entire concept is in need of more appropriate attack, taking advantage of newer methodologies of enzymology and immunology to evaluate its scientific validity.

The conclusion seems warranted that an hypothesis has been stated and restated by a single protagonist. It remains tenuous and implausible and requires support from critically designed experiments. At this time it is far from an established fact that the drinking of homogenized milk contributes to mortality from coronary artery disease through the intestinal absorption of bovine xanthine oxidase. To advise the public to avoid homogenized milk or to boil milk for this purpose, on the basis of this meager published evidence, is unwarranted and unjustified.

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R.E.S
draft

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Plasmalogens are phospholipids and are components of cell membranes of muscle and of the myelin sheath of nerve fibers. The plasmalogen of myocardium is a choline phosphatide, containing an unsaturated fatty acid in ester linkage and a second fatty acid as a vinyl ether (2). Aldehydic compounds or plasmals are released by action of an enzyme, vinyl etherase, on plasmalogens. The plasmal reaction, using fuchsin and sulfurous acid, permits staining of plasmalogens in tissue sections. Oster, utilizing this histochemical procedure on sections of myocardium from one patient dying acutely of coronary artery disease, was unable to demonstrate plasmalogens (3) and later reported absence of plasmalogens in fatty streaks of the aorta of a young drowning victim (4). From these observations deriving from the two autopsies, he concludes that plasmalogens disappear from myocardium promptly after infarction and are not present in the earliest lesions of atherosclerosis. Other studies which have determined the quantity of aldehydogenic plasmalogens after extraction from several hundred human aortas, report a decrease with age and with the severity of the atherosclerotic

lesion (5,6). These reports do not include observations on the plasmalogen content of the myocardium.

In 1944 Oster had observed that bovine xanthine oxidase would oxidize plasmal aldehydes in vitro (7). Since normal myocardium contains little or no xanthine oxidase and since in human's greatest activity of this enzyme is found in liver and intestinal mucosa, he reasoned that perhaps the enzyme might play a role, if it came from an extrinsic source, (such as in milk consumed as food), was absorbed and deposited in active form in the myocardium. In the effort to demonstrate a possible role for xanthine oxidase, activity of the enzyme was determined in the aortas of four patients aged 54 to 75 years with atherosclerotic disease and in the myocardium of two of these (8). Similar studies were done on the aorta of a 37 year old man without obvious atherosclerosis, who died of trauma. Oster reports that elevation in xanthine oxidase activity was found only in the aorta and myocardium when there was gross evidence of atherosclerotic change. However, the analyses are few in number and descriptive information is limited. No effort was made to characterize the enzyme as having bovine or human origin.

Still another line of argument is offered to support the role of bovine xanthine oxidase. Oster sees in the homogenization process, the likelihood of more ready absorption of the enzyme. Since xanthine oxidase is bound to lipoid globules in milk and homogenization increases the proportion of such globules of small size (1 micron or less), he believes that there is greater likelihood of intestinal absorption of the intact enzyme. He adds that death

rates from coronary artery disease have increased in the U.S. since introduction of the homogenization process in the 1930's, and that populations in other countries who characteristically consume boiled or curdled milk do not have high mortality rates. These arguments fail to take cognisance of the facts that heart disease mortality rates began their increase well before there was widespread use of homogenized milk in this country and that rates have been decreasing in the last decade.

Other carefully designed experiments have brought conclusive evidence that protein macro-molecules may be absorbed in the intestine of experimental animals in biologically and immunologically active forms and quantities (9). These experiments have involved proteins with molecular weights of 80,000 or less. In contrast, the bovine xanthine oxidase molecule has a weight approximating 300,000. Crucial to the hypothesis advanced by Oster is the need to demonstrate unequivocally, that xanthine oxidase in homogenized milk is absorbed intact and retains biologic activity in human tissues. An effort has been made to bring such evidence (10). Unfortunately, it is not convincing and leaves many questions concerning its scientific validity. The procedure used was a test for antibodies in human sera using a partially purified bovine xanthine oxidase as antigen in a tanned erythrocyte system. The sensitivity and specificity of the reaction has not been assessed and a crude measure of antibody potency was utilized. Blood sera were obtained on two groups of patients seen by an internist. One group of 41 patients described as a control group, was stated to have no clinical evidence of atherosclerotic disease. The second

group of 34 patients, had a variety of manifestations ascribed to atherosclerosis, i.e. angina pectoris, myocardial infarction, claudication, periferal vascular disease, cardiac arrhythmias and brain syndrome. The two groups are not comparable in age or sex distribution and the control group includes patients with hypertension and diabetes mellitus, who would be likely to have some atherosclerotic involvement. The authors claim to have demonstrated specific antibody to bovine xanthine oxidase in both groups of patients and with somewhat higher titers in the patients' atherosclerotic disease. Such demonstration, however, does not assure that the absorbed bovine enzyme is biologically active or that it is in fact deposited in the target tissues, arterial wall and/or myocardium. Under any circumstance, the entire concept is in need of more appropriate attack, taking advantage of newer methodologies of enzymology and immunology to evaluate its scientific validity.

The conclusion seems warranted that an hypothesis has been stated and restated by a single protagonist. It remains a house of cards and requires underpinning with more solid scientific bases. It is not at this time, an established fact that the drinking of homogenized milk contributes to mortality from coronary artery disease through the intestinal absorption of bovine xanthine oxidase. To advise the public to avoid homogenized milk or to boil milks for this purpose, on the basis of published evidence, is unwarranted and unjustified.

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Adopted by Nutr Com 3/20/75

Position Statement Concerning the Claims that Consumption of Homogenized Milk Increases the Risk of Heart Disease (To be distributed to staff of the American Heart Association and its affiliates. Not for press release.)

There has been and continues to be wide distribution through various public media - the press, radio and television - of claims that excessive mortality from heart disease in the United States can be attributed to widespread consumption of homogenized milk. The claims are based on several publications in the scientific literature by Dr. Kurt Oster of Park City Hospital in Bridgeport, Connecticut. These purport to demonstrate that there is increased xanthine oxidase activity and decreased plasmologen content of coronary arteries and myocardium in hearts of men dying of coronary artery disease. It is suggested by Dr. Oster that the xanthine oxidase derives from food sources, particularly homogenized milk.

The Nutrition Committee of the American Heart Association has reviewed the scientific evidence and finds it very limited. Certainly it is not sufficient to justify the claims of Dr. Oster that xanthine oxidase of homogenized milk is absorbed and then deposited in its physiologically active form in coronary arteries and myocardium. A cause and effect relationship between homogenized milk and coronary artery disease has not been demonstrated. Therefore, at this time it would seem to be most appropriate to identify these claims as yet another of

many hypotheses concerning the etiology of coronary artery disease. It is the judgment of the Committee that there is no justification at this time for recommendations to the public for avoidance of dietary intake of homogenized milk for the reasons given by Dr. Oster.

By CHARLES B. MICHELINI

Leading Specialist Warns: Milk Is a Major Cause Of Heart Disease Deaths

Homogenized milk — the common form of milk sold everywhere in America — is a killer causing death from heart disease on a wholesale scale, warns a leading cardiac specialist.

"Homogenized milk is one of the major causes of heart disease in the U.S.," reports Dr. Kurt A. Oster, who pioneered the research leading to his startling announcement.

And the head of Harvard Medical School's Dept. of Medicine, along with other specialists, agrees with Dr. Oster's shocking conclusion.

Homogenized milk is milk that has been processed to break up its fat content into very tiny particles, so that they are evenly distributed. This prevents it from separating into cream and skim milk when it stands, as non-homogenized milk will do.

But, Dr. Oster told The ENQUIRER, the fat in milk contains a substance called xanthine oxidase, or XO, an enzyme. This enzyme will attack the heart and its arteries if it enters the bloodstream — and it is able to get into the blood from homogenized milk.

"When old-fashioned, non-homogenized milk is drunk, the body excretes the XO like any other waste," he said.

"But when milk is homogenized, the breakup of the fat allows the tiny particles of XO to go through the walls of the intestine into the bloodstream and reach the heart and artery tissues," explained Dr. Oster, chief of cardiology at Park City Hospital, Bridgeport, Conn.

The XO acts chemically to scar the artery walls and heart tissue, he said. The body tries to repair the damage by raising the cholesterol level of the blood, and depositing protective fatty material on the scars. If the process continues, the fatty material begins to clog the arteries, causing heart disease.

However, he stressed, anyone can easily remove or greatly reduce the XO danger from homogenized milk — simply by heating the milk (see box at right).

Said Harvard Medical School's Dr. Kurt Esselbacher, chairman of its Dept. of Medicine: "I am in full support of Dr. Oster's overall concept. Homogenized milk, because of its XO content, is one of the major causes of heart disease in the U.S."

Heart disease is the greatest single cause of death in the U.S. An estimated 1,054,500 Americans will die of heart and heart-related disease this year, according to the American Heart Assn.

"Homogenized milk is the main reason why the U.S. cardiac death rate is the highest in the world, next to Finland's," declared Dr. Oster. "The process became routine in the U.S. about 1938. But it does not improve the taste or nutritional value of milk. All it does is prolong its shelf life, increase its cost, and cause a lot of damage in the body." Skim milk and ice cream sold in the U.S. are also homogenized, according to the American Dairy Assn.

In his first studies, made in 1971 with Dr. Donald J. Ross, professor of biology at Fairfield College, Conn., XO was found in the heart and artery tissues of five people who had died from heart disease.

"Normally, XO shouldn't be there at all," said Dr. Ross. "In addition, we found that these people were heavy milk drinkers."

Said Dr. Oster: "From 1971 to 1974, we studied 75 patients with angina pectoris" — chest pain due to heart disease — "and atherosclerosis" — hardening of the arteries.

"All the patients were taken off milk and given folic acid (a B vitamin) and ascorbic acid (Vitamin C)" — both of which combat the action of XO.

"The results were dramatic. Chest pains decreased, symptoms lessened, and each one of those patients is doing great today."

Dr. R. Lincoln Kesler, chief attending physician at Rush-Presbyterian-St. Luke's Medical Center in Chicago, is another who says homogenized milk is a killer. "The first thing I do with heart patients is take them off homogenized milk," he said.

Dr. Oster claimed that the foundations of heart trouble start early in most Americans, because children drink so much milk.

"The damage caused by XO is a long-term process," he said. "The XO builds up in the body. The first 10 to 15 years, when most children drink a lot of milk — that's when the real damage is done."

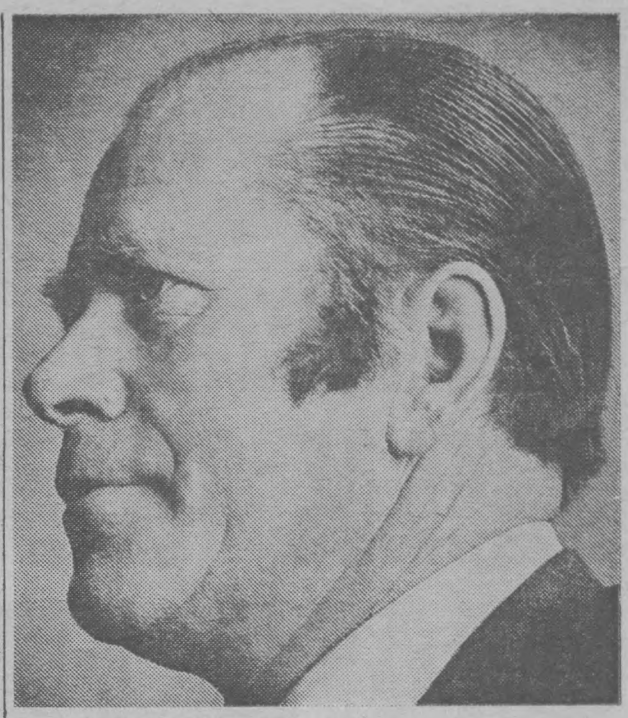
How You Can Remove Deadly Effects of Milk

You can, in your own home, completely remove or greatly lessen the dangerous effects of XO from homogenized milk, says Dr. Kurt A. Oster.

"If Americans will simmer their milk we could cut our death rate from coronary disease in half," he claims. "Heat kills the XO." To simmer milk, heat it until it is just below the boiling point, when tiny bubbles are beginning to form at the edge of the pan. It can then be cooled and put in the refrigerator.

Dr. Oster claims the dairy industry could remove the danger of XO from all homogenized milk by a change in its pasteurization process — the method used to kill bacteria. Pasteurization is separate and apart from homogenization.

"The milk industry now pasteurizes milk by heating it to 173 degrees, and holding it there for 19 seconds," he said. "But that destroys only about 30 percent of the enzyme's activity. If they would raise the temperature just 10 degrees it would completely kill the XO."



Would You Believe It's Not President Ford?

It's Robert Smeding, a 49-year-old interior decorator of Penticton, British Columbia. The Canadian, who's 2 inches taller and 20 pounds lighter than President Ford says he hasn't had a "hello" since Gerald Ford became President. Strangers look at him quizzically and say, "President Ford?" Smeding added, "I'm proud to look like Mr. Ford, but I sure don't want anyone taking a shot at me."

15% of Migraine Sufferers Are Under 10 Years Old

About 15 percent of all migraine headache sufferers are under 10 years old, says a pediatrician. Boys and girls are affected equally until adolescence, when the incidence of migraine in girls rises, said Dr. Marvin Klein, of New Hyde Park, N.Y.

Addressing members of the American Academy of Pediatrics, he said signs of migraine are believed by many to be detectable as early as age 2.

Ringo Starr: My Marriage Is Over— I've Got a New Girl

The 10-year-marriage of Ringo Starr to the girl next door is on the rocks and the ex-Beatle is off and running with a brand new girl.

"My marriage is over," Ringo said, arriving at Los Angeles Airport from London. "I'll be going back to Britain one day, but I won't be going back to Maureen. Nancy is my girl now."

Ringo was talking about 24-year-old Nancy Andrews, a Hollywood model, who was hanging on his arm.

"I'm no marriage-breaker," Nancy interrupted. "Ringo's marriage was over 18 months before I met him. We got on well at first and still do.

NEW ROMANCE: Ringo Starr with Nancy Andrews at a party. Says Ringo: "She's a nice lady who looks after me."

"And our feelings for each other have grown.

"We communicate on the same level — we get on. It's a great romance and I now see myself being with Ringo for a long, long time."

Ringo, who's 34, married Maureen in February 1965.

While he gallivants around the world with his new love, his wife is in England with the couple's three children.

"Nancy's a nice lady who looks after me. And I enjoy being looked after," said Ringo, dressed in a black suit, a velvet-collared black overcoat and a silver cross earring.

But he skirted questions about divorce and remarriage.

"I'm a married man with three children," he said. "I can't talk about getting married now. I suppose my marriage — like all the marriages I know — will end in divorce.

"But for the moment I'm not starting divorce proceedings.

"I'm not saying my marriage will end in divorce, but that seems to be the way things go today.

"And I'm a today person — so draw your own conclusions."

Ringo said Nancy hasn't met his wife, Maureen.

"I don't want them to meet," he said. "They're different people and this is a different phase of my life."

And he added that he doesn't plan to introduce them even though he's planning to take Nancy with him when he returns to England.

— *ALAN MARKFIELD*

NATIONAL ENQUIRER

March 11, 1975; Vol. 49, No. 30
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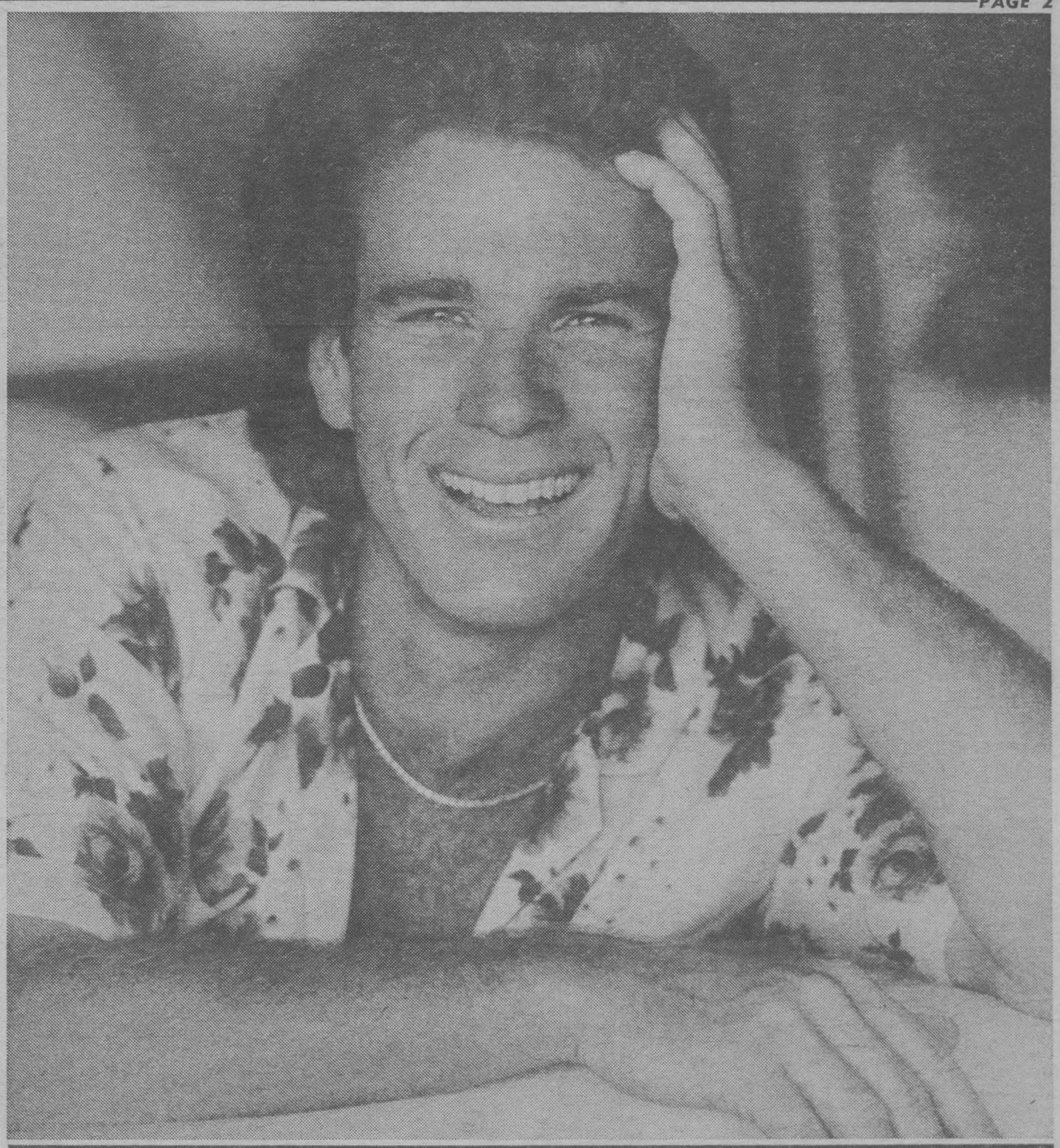

Ryan O'Neal: I Can't Be Faithful to One Woman

In his frankest interview ever, Ryan O'Neal declares: "I'm the greatest father in show business . . . but I'm the worst husband in the world." The twice-divorced actor — father of Oscar-winner Tatum O'Neal and two boys — boasts: "My children are my most satisfying pleasure." (Exclusive story on Page 37.)

The Presence of Ectopic Xanthine Oxidase in Atherosclerotic Plaques and Myocardial Tissues[1] (37627)

DONALD J. ROSS, MICHAEL PTASZYNSKI,[2] AND KURT A. OSTER

Department of Biology, Fairfield University, Fairfield, Connecticut 06430; and Section of Cardiology, Department of Medicine, Park City Hospital, Bridgeport, Connecticut 06604

Plasmalogens, a naturally occurring group of aldehydogenic phospholipids, are found abundantly in human heart and brain tissues. Their phospholipid character makes them an important constituent of many biological membrane systems. Ferrans, Hack, and Borowitz (1) demonstrated plasmalogens in the sarcoplasm, sarcosomes, and intercalate discs of normal human cardiac muscle. Oster and Hope-Ross (2) examined histochemically cardiac muscle from a case of fatal myocardial infarction and found that plasmalogen had disappeared from the infarcted area less than 2 hr after the onset of pain. In this case, there was no necrosis or other significant tissue changes in the affected heart muscle. Similarly, Oster (3) demonstrated the absence of plasmalogen in the aorta of a 22-year-old drowning victim suffering from extensive atherosclerotic changes. Other investigators (4, 5) also demonstrated that plasmalogen depletion in the aortic wall corresponded to an increase in atherosclerosis and that aortic plasmalogen concentrations decreased with age.

It is of significance that certain metabolically active organs —*e.g.*, the liver and mucous membranes of the small intestine —are normally devoid of plasmalogens. Oster and Mulinos (6) ascribed this absence to the activity of the enzyme, xanthine oxidase (xanthine: oxygen oxidoreductase, EC1.2.3.2) which abounds in those tissues where plasmalogen is normally absent. These authors demonstrated that when plasmalogen was split into a fatty aldehyde and lysoplasmalogen by the action of dilute HCl, the resulting aldehydes could be oxidized by purified bovine milk xanthine oxidase preparations. This finding provides a reasonable biochemical explanation for enzyme-mediated plasmalogen depletion. Prior studies by Morgan (7) and Ramboer (8) report the absence of xanthine oxidase in normal human cardiac tissues. The present preliminary investigation endeavors to explain the plasmalogen depletion phenomenon by examining diseased human aortic and myocardial tissues for ectopic xanthine oxidase. No report of the enzyme's presence or absence in diseased tissue has been found in the literature.

Methods. Tissue Sample Selection and Preparation. Unfixed aortic and myocardial tissues of a 54-year-old male and a 74-year-old male were examined (Cases 1 and 2). Their deaths were due to the complications of an abdominal aortic aneurysm and to a myocardial infarction, respectively. In 3 additional cases, the examination for ectopic xanthine oxidase was confined only to the aorta. Case 3 was a 61-year-old male who died from the complications of a bleeding peptic ulcer. He had extensive atherosclerotic calcification of the aortic arch demonstrated by x-ray during life. Case 4 was a 75-year-old male who died from pneumonitis. Calcifications of aorta were examined. Case 5 was a 37-year-old male who died suddenly as a result of a trauma. There was no appreciable amount of atherosclerosis or arteriosclerosis in the aorta.

The entire aortic wall was dissected into little tissue squares (1.0 cm^2) visibly containing yellowish atherosclerotic plaques and similar squares of apparently normal, less involved pinkish aortic tissue. The decision for

[1] Supported in part by a research grant from the Greater Bridgeport Chapter of the American Heart Association.

[2] Present address: Hahnemann Medical College, Philadelphia, Pennsylvania.

the myocardial sampling was more difficult because of the lack of a clearly visible demarcation between normal and pathological tissue. Sections near the anterior coronary artery branch were compared with apparently more normal-looking tissue closer to the apex of the heart. Grossly, the latter area showed no evidence of scarring. An average of 8 enzyme assays were performed on homogenates of 2–4 tissue samples from each person.

Estimation of Xanthine Oxidase Activity. Xanthine oxidase activity was measured by the method of Haining and Legan (9). Aortic and myocardial tissue samples were homogenized in 10 vol of cold 0.05 *M* phosphate buffer (pH 7.4) with EDTA. The homogenate was then centrifuged for 30 min at 4° and 48,000*g*. The supernatant liquid containing the enzyme was subsequently passed through a Sephadex column (K 9/30) containing G-100 gel which had been preirrigated for 1 hr with 0.1 *M* phosphate buffer (pH 7.4) without EDTA (8). When present, the enzyme would appear in the eluate.

For assay, the reaction mixture consisted of 2.7 ml of 2-amino-4-hydroxypteridine (AHP) in 0.2 *M* phosphate buffer (pH 7.4) as the substrate and 0.3 ml of the enzyme solution. Appropriate fluorometric blanks were prepared by incubating the buffer substrate and the enzyme solutions in separate test tubes and then combining them after the incubation period and just prior to reading. These blanks and the reaction mixtures were incubated for 1 hr at 37°. Following incubation, 3 ml of impurity-free 40% trichloracetic acid were added to 1 ml portions of both blanks and reaction mixtures. The resulting turbid mixtures were then centrifuged for 10 min at 10,000*g* to remove precipitated protein. The supernatant fluorescence was measured in a Beckmann Ratio Fluorometer using a number 5 uranium bar, a Schott UG-11 primary filter, and a second Wratten 2A filter. A quinine sulfate solution (1.6 *M* in 0.1 *N* sulphuric acid) served as the 100% fluorescence standard.

The unit of enzyme activity was established by the Haining and Legan method, so that each fluorometer scale division measured is equivalent to 1.26×10^{-4} μmoles of AHP oxidized to the fluorescent product, isoxanthopterin (9). The unit of enzyme activity is expressed as the number of moles of AHP oxidized per g of tissue per hr.

Results. The results (Table 1) indicate the presence of xanthine oxidase in many of the samples investigated. As shown, the lowest values for enzyme activity (≤ 4.69) were observed in aortic tissue samples which appeared grossly normal. Significantly ($p < 0.01$) higher activities were found in samples from both atherosclerotic aortas as well as the pericoronary and apical myocardial tissues of both heart specimens. The variations among the high readings can be ascribed to the differences in severity of the sample pathology, since it is known that tissue reactivity in atherosclerosis and myocardial damage is uneven.

In Cases 3–5, where just the aorta was examined, only one (Case 3) showed ectopic xanthine oxidase in an atherosclerotic lesion. Case 4 with a history of liver disease had no detectable ectopic xanthine oxidase in the lesions examined. The atherosclerotic process in this case showed the severest degree of calcification. Case 5, whose aorta was essentially normal, exhibited no detectable ectopic xanthine oxidase.

Discussion. For the first time, to our knowledge, the presence of xanthine oxidase in the atherosclerotic plaque and the pathological myocardium has been demonstrated. Grossly normal aortic tissues exhibit very little or no detectable enzyme activity, and the same has been reported for normal heart muscle (7, 8). It is possible that this ectopic xanthine oxidase may encounter a suitable substrate in the aldehyde moiety of the phospholipid plasmalogen which is a normal constituent of the cell membranes of such tissues. The subsequent alteration of the structural integrity of these membranes by such enzymatic activity may produce an initial lesion which could then serve to increase cell permeability, microthrombus deposition, or both.

One source of the enzyme may be the liver cell, since patients with acute liver disease show increased serum levels of xanthine oxidase (8). In patients with chronic liver disease, the serum level of xanthine oxidase

TABLE 1. Xanthine Oxidase Activity in Normal and Pathological Human Aortic and Myocardial Tissue Homogenates.

Male patient no.	Age	Tissue sampling	Samples (n)	Mean g tissue/ml homogenate	Mean moles AHP[a] oxidized/hr	Xanthine oxidase activity[b]
1	54	Normal-appearing aorta (control)	3	0.329	$1.89 \times 10^{-4} \pm 0.04$	4.69 ± 0.1
1		Atherosclerotic aorta	4	0.493	$5.56 \times 10^{-3} \pm 0.05$	89.5 ± 0.8
2	74	Normal-appearing aorta (control)	2	0.289	$<5.6 \times 10^{-5}$	
2		Atherosclerotic plaque (aorta)	3	0.239	$1.01 \times 10^{-3} \pm 0.07$	33.5 ± 2.3
1		Normal-appearing myocardium (control)	2	0.220	•	
2		Normal-appearing myocardium (control)	2	0.244	•	
1		Pericoronary myocardium of right lateral branch of coronary artery	2	0.301	$2.45 \times 10^{-3} \pm 0.04$	65.3 ± 1.5
2		Area from apex of heart with no visible epicardial scarring	3	0.332	$1.10 \times 10^{-3} \pm 0.03$	26.3 ± 1.4
3	61	Atherosclerotic aorta	3	0.219	$7.02 \times 10^{-3} \pm 0.14$	28.19 ± 2.7
4	75	Atherosclerotic aorta	3	0.314	•	
5	37	Normal aorta (control)	3	0.219	•	

[a] Moles of 2-amino-4-hydroxypteridine (AHP) oxidized g tissue hr. An average of 3 enzyme assays were performed on each tissue sample. Results are expressed as averages ± SD.

[b] Minimal detectable activity level for method employed.

is occasionally moderately elevated. Moreover, in uncomplicated, obstructive jaundice, the serum xanthine oxidase is, at times, slightly elevated (8). In addition to these findings, Ramboer (8) was able to demonstrate slight xanthine oxidase activity in the sera of 10 out of 25 normal human subjects, although Shamma'a *et al.* (10) detected no xanthine oxidase activity in the sera of 18 healthy subjects. Another potential source of the enzyme, *viz.* bovine milk ingestion, is presently under investigation in this laboratory, since it has been shown that milk antibodies are significantly elevated in the blood of male patients with ischaemic heart disease (11).

The postulated enzyme-induced alteration of the phospholipid composition of the cell membrane may point to ectopic xanthine oxidase as one of the factors inducing serious inflammation or perfusion of the arterial endothelium or the myocardium as described by Haust (12). Roussos (13) has shown that bovine xanthine oxidase activity is stimulated by androsterone and testosterone and inhibited by the estrogens (β-estradiol, 17α-estradiol, estrone, and estriol) and progesterone. This could account for the predominance of atherosclerotic heart disease in men.

Summary. Xanthine oxidase activity of five grossly normal aortic tissues of five male patients was compared with that of atheromas from the same aortas. In addition, pathological myocardial tissues of two of the patients were examined. Significantly elevated enzyme activities were found in most abnormal tissue samples. Little or no activity was detected in the normal-appearing samples. These results suggest that xanthine oxidase may be deposited gradually with time, possibly initiating a pathological reaction which culminates in plaque formation or myocardial cellular damage.

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CNI

Newspaper Story Sparks Homogenized Milk Flap

The National Enquirer, a tabloid newspaper sold at supermarket checkout stands, sparked a major nutrition controversy this month with publication of an article attacking homogenized milk.

"Milk Is a Major Cause of Heart Disease Deaths... But There's a Simple Way You Can Make It Safe" read a banner headline on the March 11 issue of the newspaper, which is normally seen by supermarket shoppers as they stand in line to pay for groceries. A smaller headline above the banner read, "Harvard Medical School Chief Backs Doctor's Warning."

The story inside the issue recounted the theories of Kurt A. Oster, a Bridgeport, Connecticut cardiologist who believes that when milk is homogenized, xanthine oxidase (XO), an enzyme found in milkfat, is able to pass directly through the digestive system into the arteries. Once in the arteries, Oster contends, XO damages the artery walls, which leads to formation of scar tissue, a buildup of cholesterol on the scars, and eventual clogging of the arterial passages (atherosclerosis). Housewives should simmer milk at a temperature just below boiling to destroy the XO, Oster advises.

"If Americans will simmer their milk we could cut our death rate from coronary disease in half," he told the National Enquirer. "Heat kills the XO."

Quote Disavowed

To back the Oster thesis, the newspaper quoted Kurt Isselbacher, chairman of Harvard Medical School's Department of Medicine: "I am in full support of Dr. Oster's overall concept. Homogenized milk, because of its XO content, is one of the major causes of heart disease in the U.S."

Unfortunately for the National Enquirer story, Isselbacher has formally disavowed these remarks and asked the newspaper to print a correction. Isselbacher's office told CNI Weekly Report that he was "surprised and shocked" to learn of the article.

"This quotation was totally incorrect," Isselbacher informed the Enquirer through his lawyer. "Dr. Isselbacher has never spoken to the reporter involved, nor has he made such a statement to anyone."

The newspaper, meanwhile, is sticking to its story, claiming that Isselbacher did, indeed, make the remarks attributed to him to a free-lance writer whose notes became the basis of the March 11 article. An Enquirer spokesman told CNI Weekly Report that the newspaper had been offered the XO story several months ago and had assigned a staff rewrite man to follow up on it. The rewrite man, whose byline appears above the article, used the Isselbacher quote but did not check it, the newspaper concedes.

The story has become the focus of a heated dispute between the newspaper and the National Dairy Council, which has been besieged with inquiries about homogenized milk since it appeared. Accusing the Enquirer of sloppy journalism, the Dairy Council has sent a point-by-point rebuttal to newspaper and magazine science writers across the nation.

"There are no published data to support the opinion that XO can reach the circulatory system in its original form," declared M. F. Brink, Dairy Council president, in a rebuttal statement.

For his part, the Enquirer spokesman called the Dairy Council attacks on the XO story "vicious" and hinted darkly of legal action. "When we do a medical-scientific story, we demand outside backup, and we got it in this case," he declared.

Dairy Council Rebuttal

Meanwhile, the controversy over homogenized milk is no nearer to being resolved. The Dairy Council suggests that Oster, having failed to win the backing of established research organizations for his thesis, is peddling it through the popular press and through forums set up by industries interested in protecting their own products from association with heart disease. (Oster has spoken at meetings sponsored by a water-softener manufacturer and by the National Commission on Egg Nutrition, an egg industry group. He told CNI Weekly Report he had volunteered his services to the egg people after reading their ads denying a link between egg consumption and heart disease risk -- see CNI Vol. IV:5.)

Oster, who is now retired as chief cardiologist at Park City Hospital in Bridgeport, contends that both epidemiological statistics and clinical research back up his XO theory of heart disease. Only widespread consumption of homogenized milk, he claims, could account for the high incidence of heart disease in the U.S. and Finland, two countries where homogenization is well-established, and the relatively low incidence in other European countries, where it is not. "All (homogenization) does is prolong milk's shelf life, increase its cost, and cause a lot of damage in the body," he told the National Enquirer.

Oster has tried without success to interest the HEW's National Heart and Lung Institute (NHLI), a branch of the National Institutes of Health, in his theory. The NHLI informed him six years ago it had assigned "low priority" to the thesis.

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"Ralph Nader and I have been impressed by the quality of your investigative journalism in CNI Weekly Report" -- Harrison Wellford, chief counsel to Senator Philip Hart.

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In New York, when it came time to balance the city budget, the city's Bureau of Nutrition was virtually eliminated as a way to save money. Catherine Cowell, director of the 30-year program, fought back and won a reprieve. She now says that nutritionists and other professionals in the food and nutrition field must be more aware and more actively involved in the legislative process.

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CNI Weekly Report covers all these areas--from the quality of food in the school lunch program and the inadequate diet provided by the food stamp program to debates over the sugar content of processed foods and research on the need for more fiber in the American diet. We make a special point of covering developments in Congress and the executive agencies on food and nutrition issues so that our readers can register a timely impact on the policy-making process.

In addition, we travel to universities and research laboratories to cover new events in nutrition research. In coming months, for example, we expect to report on some highly significant and previously unpublished information from the Ten-State Nutrition Survey, and what several leading nutrition researchers believe the new information means.

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Sincerely,

Rod Leonard

Rodney E. Leonard
Executive Director

PLASMALOGEN DISEASES: A NEW CONCEPT OF THE ETIOLOGY OF THE ATHEROSCLEROTIC PROCESS

Kurt A. Oster, M.D.*

A new disease entity called Plasmalogen Disease is proposed. It originates from the oxidation of the plasmal aldehydes of plasmalogen by ectopic xanthine oxidase. This enzyme may be absorbed by ingestion, especially of the micro-sized droplets found in homogenized bovine milk. It is found in atherosclerotic plaques in which the amount of normally present plasmalogen is greatly diminished. Similar observations can also be made in the myocardium. Fatty streaks, the precursors of atherosclerosis, are early manifestations of plasmalogen disease. A comparison of death rates from myocardial infarction with the consumption of dairy products in various nations shows no correlation whatsoever with the intake of saturated fatty acid but a very close fit with the intake of biologically available xanthine oxidase. A simple method for the primary prevention of atherosclerosis in youth would be the creation of a biologically available xanthine oxidase-free milk. Interim steps recommended would be the cessation of homogenization and/or preheating of milk by the consumer.

In 1958 the World Health Organization introduced a system of classifying atherosclerotic lesions, starting the sequential process with those lesions, already found in youth, designated as "fatty streaks."[1] Placing the initiation of the atherosclerotic process in the period of adolescence has met increasing acceptance. Since these changes are evident at such an early age, it is difficult to reconcile the genesis of the fatty streak with such frequently mentioned risk factors as hypertension, obesity, cigarette smoking, lack of physical activity, and emotional stress-provoking situations. The findings of Enos *et al*[2] that 12% of American soldiers killed in the Korean War exhibited 50% occlusion of the coronary lumen, and 5% had 90% occlusion, speak against the theory of hypercholesterolemia and electrocardiographic abnormalities, which conditions were not present in these young men. Similar findings were reported in a Chilean study.[3]

Even if one does not equate the presence of this excessive amount of coronary occlusion with later manifestations of ischemic heart disease and eventual myocardial infarction, one must admit that a severe degree of atherosclerosis can exist in young people. Are we not then justified in questioning the cause of these fatty streaks in the adolescent who exhibits no trace of hyperlipidemia? Conversely, the temptation is great to doubt the vigorously promoted theory that hyperlipidemia is the cause of these lesions and that primary prevention directed to reducing the cholesterol level in blood serum by dietary manipulation may be based on false premises.

The hyperlipidemia theory of atherosclerosis has been in existence for more than fifty years. Because it attributed the origin of atherosclerosis to an increase of various fatty substances in the plasma, it has proposed prevention of the condition by such dietary changes as low cholesterol and normal fat, low fat, normal cholesterol and lower saturated fats, and increase in polyunsaturated fats. This has spawned a deluge of animal experiments designed to mimic human pathology, and much of the work has caused confusion by creating conditions of unreality and by producing strange diseases which do not fully simulate human atherosclerosis. If we free ourselves from this hyperlipidemia theory, we might then postulate that the etiology of atherosclerosis lies not necessarily in an overabundance of such fatty substances as cholesterol, triglycerides, or lipoproteins, but rather in a deficiency of defined chemicals localized in the arterial intima, as the first step, and the infiltration

*Chief, Section of Cardiology, Park City Hospital, Bridgeport, Conn.

Portions of this paper were presented at the 3rd Annual Meeting of the International Study Group for Research in Cardiac Metabolism, Stowe, Vt., June 29-July 1, 1970.

by cholesterol esterified with unsaturated oleic acid[4] in the depleted area, as the sequela. This infiltration might be described as the body's repair mechanism to the original noxa.

The various plasmalogens, chemicals normally found in the arterial intima and also in cardiac muscle tissue, fulfill admirably the requirements of this deficiency theory of the onset of atherosclerosis. For the last thirty years the author has marvelled at the selectivity of the histochemical localization of the plasmalogens. Their eventual biologic significance in human metabolism has been studied in both animal and human experimentation.

Since plasmalogens are unfamiliar to many clinicians, the following may serve as a brief introduction to their chemical structure. Plasmalogens are polar substances related to lecithin, in the older terminology, or phosphatidyl choline, in the newer nomenclature. One of the plasmalogens is an enol ether of either palmitic or stearic aldehyde attached to a lysoplasmalogen: $CH_2OCH{=}CHR^1$

$$\begin{array}{l} RCOOCH \quad O \\ \qquad\quad \| \\ CH_2OPOCH_2CH_2N\,(CH_3)_3 \\ \qquad\quad | \\ \qquad\quad O \end{array}$$

R^1 = Fatty aldehyde; R = Unsaturated fatty acid

Cleavage of the aldehyde moiety from the lysoplasmalogen may be effected in histochemical examination by $HgCl_2$ or other heavy metal salts, by weak acids, or, biologically, by the enzymes phospholipase A and vinyletherase, which are present in many tissues. Plasmalogen aldehydes are called plasmal. Choline plasmalogens are abundantly present in heart muscle, whereas in the myelin sheath of the central nervous system colamine or serine plasmalogens prevail. There are also many species variations in plasmalogen chemistry. Nevertheless, it has been stated that *up to 30% of the phospholipids found in heart muscle are plasmalogens,* especially those situated in the cell membrane. One wonders why so little is known about the biological significance of these substances which have such wide distribution in vital tissues.

Anatomical location of the plasmalogens has been thoroughly reviewed by Oster.[5] In addition to the arterial intima and the myelin sheath of nerve tissue, plasmalogens are found in certain portions of the kidney, mucous membranes of the large intestine, the adrenal cortex, and many other distinct organs. It is of great interest that certain metabolically active organs are devoid of plasmalogens, namely, the liver and mucous membranes of the small intestine. The absence of plasmalogen from these anatomical sites was ascribed by Oster and Mulinos[6] to the activity of the enzyme *xanthine oxidase, which is present in abundance in those locations where plasmalogen is absent.* It was shown at that time that when plasmalogen was split into aldehyde and lysoplasmalogen by the action of dilute HCl, the resulting aldehydes could be oxidized by xanthine oxidase derived from milk

Plasmalogens were shown to be target substances for the action of male and female sex hormones. Oster[7] proved that the distribution of these chemicals in the female rat kidney was governed by the phases of the estrous cycle. It was demonstrated that the disappearance and reappearance of plasmalogens in the rat kidney could be duplicated in the castrated animal by the administration of male or female hormones. These changes, should they be shown to occur also in the human organism, might possibly explain the lower incidence of myocardial infarction in women of child-bearing age than in men of equivalent years.

Oster and Hope-Ross[8] performed a histochemical examination of cardiac muscle from a case of fatal myocardial infarction and found that plasmalogen had disappeared from the infarcted area less than two hours after the onset of pain. There was no necrosis or other significant tissue changes of the affected heart muscle. In effect, plasmalogen leaked out of the infarcted region *before* any other demonstrable changes occurred. Surely this substantiates the theory that plasmalogens are associated with

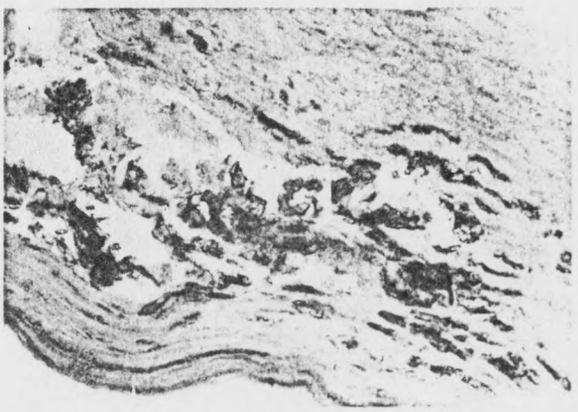

Figure 1. Aorta of a 22 year old apparently healthy drowning victim. Fuchsin sulfurous acid plasmal reaction × 480. Plasmalogen has disappeared from behind the intact intima. The darkish material infiltrating the area where plasmalogen has disappeared most likely consists of cholesterol esterified with monounsaturated oleic acid.

myocardial damage.

Similar absence of plasmalogen was found in the aorta of a 22 year old drowning victim suffering from extensive atherosclerotic changes of this blood vessel (See Fig. 1). Buddecke and Andresen[9] also have shown that increased plasmalogen loss from the aortic wall corresponds to an increase in atherosclerosis.

Making use of the described observation that xanthine oxidase destroys the aldehydes liberated from plasmalogen.[6] Oster attempted to restore depleted plasmalogens to the myocardium by inhibiting xanthine oxidase action with a known xanthine oxidase inhibitor, allopurinol.[10] This clinical effort, which was undertaken in sixteen patients suffering with angina pectoris, met with gratifying results. A dose-effect relationship could be established between the quantity of allopurinol and the reduction of nitroglycerin needs. It was then postulated for the first time that angina pectoris and myocardial infarction may be *plasmalogen diseases* and that manifestations of plasmalogen depletion might also be found in other histochemical sites, *e.g.,* in the myelin sheath in multiple sclerosis.[11]

Such a plasmalogen depletion could occur if there existed an overabundance of xanthine oxidase, or if the activity of this enzyme could be stimulated, or if it were mobilized from its storage position in the liver and small intestine,[6] thereupon to exert its aldehyde-oxidizing power where the presence of these aldehydic substances is vital and necessary for the persistence of cell equilibrium or biological functioning. Milch's studies have proven the presence of aldehydes to be essential for the maintenance of elasticity in the arterial wall.[12] Also, Oster has postulated that tissue aldehydes may serve as receptor substances for certain amines and other pharmacologically active radicals.[13] If enough of these biologically essential aldehydic substances should be oxidized by excess xanthine oxidase, a pathological deficiency would result which the body might try to rectify. Such corrective steps may take the form of lipid infiltration,[4] as witness the fatty streak in the arterial wall, or eventual tissue necrosis, as seen in the myocardium.

In the following the author will show how xanthine oxidase may enter the blood stream by ingestion. The only food containing a significant amount of this enzyme is bovine milk. The milk industry in this country pasteurizes milk at 170°F. with a fifteen second holding period, and this practice leaves about 42% of the xanthine oxidase in its active state.[14]

One of the basic tenets of pharmacology states that particle size determines rate of absorption into the body, and micronization of particles facilitates their entry into the blood stream from the intestine.[15] Xanthine oxidase in milk, a part of the microsomal particle, is situated in the fat globules, and for the past thirty years the size of these fat droplets has been altered, micronized, "homogenized." Homogenization of milk became a routine practice in the United States about 1938.

"The globules of normal cow milk vary in size from about 0.1μ to about 15μ in diameter, averaging about 3.5μ with 80% in the 2.0 to 5.0μ range . . . When milk is homogenized, the fat globules are broken up and reduced in size from an average diameter of 3.5μ to an average of about 1μ. This increases the number of globules some one hundred times and expands the fat globule surface between six and seven times."[16]

Reimann in his studies of microcrystal absorption has proved that completely insoluble crystals of non-absorbable material do, when present as microcrystals, penetrate through the intestinal mucosa and are transported through the microvilli into the venous and the arterial blood, via the lymph system, and are found deposited in several body locations, including the myocardium.[17]

It is then the smallness of the particle size which creates a completely new aspect of pharmacological behavior. Microcrystals attain blood levels not usually accomplished by the regular crystal size of such drugs as griseofulvin.[18] Thus, homogenization of milk, creating micronized fat globules, will enhance the absorptive potential of xanthine oxidase through the intestinal mucosa whence it eventually reaches the blood stream to become biologically available.

If Koch's three postulates for establishing the presence of an organism causing a specific infection were to be applied to the concept of plasmalogen disease caused by xanthine oxidase of dietary origin, one would have to prove its presence in the food, its presence in the pathological lesion and its absence from normal tissue, and, finally, the reproducibility of the lesion in other species. The first two points have been investigated by Ross, Malts and Oster,[19] and by Ross, Ptaczinski and Oster[20] respectively. Their examination of milk products obtained from a dairy and grocery shelves demonstrated the presence of xanthine oxidase, especially in homogenized milk and even the so-called 99% fat-free milk, also homogenized. Exam-

ination of autopsy material from two men, 54 and 74 years old, clearly demonstrated the presence of xanthine oxidase in the atherosclerotic plaque and its almost complete absence from adjacent normal tissue. Similar findings were obtained from cardiac muscle tissue.

Xanthine oxidase, then, satisfies two of Koch's three postulates. To produce atherosclerosis in experimental animals by the ingestion of xanthine oxidase one must assume that the animals have the same intestinal absorptive capabilities as do humans, and this is most unlikely. The administration of xanthine oxidase to humans on an experimental basis is impracticable, since tissue results can be studied only in autopsy material.

However, Koch's third postulate can be satisfied retrospectively by studying the milk consumption of large population groups, comparing the intake of biologically active xanthine oxidase-containing milk products with the published frequency of death from arteriosclerotic and degenerative heart disease. Table I is such a comparison.

TABLE I

Country	1967 Death Rate	Pounds Per Person Fluid Milk Intake	Pounds Per Person Butter	Pounds Per Person Cheese	Relative Standing M	Relative Standing B	Relative Standing C	Homogenized	Pre-Boiled Frequently
1. Finland	244.7	593	35.	7.3	1	1	12	1/3	No
2. United States	211.8	273	4.7	10.6	9	11	7	almost all	No
3. Australia	204.6	304	22.9	7.8	7	2	11	not general practice	
4. Canada	187.4	288	16.2	9.0	8	8	9	partially	No
5. United Kingdom	140.9	350	19.7	11.0	4	4	6	about 7½%	No
6. Netherlands	106.9	337	5.7	19.5	5	10	4	infrequently	
7. F.R. of Germany	102.3	213	18.7	9.3	11	5	8	partially homogenized	
8. Austria	88.6	327	13.2	8.4	6	9	10	occasionally homogenized	
9. Sweden	74.7	374	16.3	18.3	2	7	5		Yes
10. Italy	78.9	137	4.0	19.9	6	12	3	12.5%	Yes
11. Switzerland	75.9	370	16.4	22.1	3	6	2	small quantity	Yes
12. France	41.7	230	19.9	28.8	10	3	1	negligible	Yes
13. Japan	39.1	48	?	?				occasionally	

Comparison of Death Rates per 100,000 Population from Arteriosclerotic and Degenerative Heart Disease with Consumption of Milk and Dairy Products.

Death rates (45-54 years old). Source: World Health Statistics Annual, World Health, Aug.-Sept. issue p. 11, 1970.

Consumption of fluid milk and cream, butter, and cheese in selected foreign countries, 1968. Source: Butz, W.T., How Americans Use their Dairy Foods, National Dairy Council, Chicago, Ill., p.15, 1970. Data on homogenization and customs obtained from the respective national dairy institutes. The cooperation of those in charge is gratefully acknowledged.

We find that Finland has both the highest death rate from heart disease and the highest rate of milk consumption. The United States, with the second highest death rate, has a relatively low milk intake. However, most milk in this country is homogenized, and homogenization multiplies the biological availability of xanthine oxidase by at least 3.5, making it 3.5 times as great as that found in non-homogenized milk. U.S. milk consumption can thus be regarded as 273 lbs. x 3.5 (homogenization factor), or the equivalent of 955.5 lbs. per person of pasteurized unhomogenized milk. The Finns surpass this figure, for according to data received about one-third of their milk is homogenized, giving them an approximate equivalent consumption of 1086 lbs. of xanthine oxidase-active milk.

The table shows that Australia and Canada present high death rates from cardiovascular disease yet occupy seventh and eighth places respectively in their fluid milk consumption. Here the questions of homogenization and preboiling are not completely resolved from the information received from their national dairy institutes. However, those countries which report a low mortality from cardiovascular disease, less than one-half that of Finland and the United States, frequently have the custom of preboiling milk before consumption, thereby biologically inactivating its contained xanthine oxidase. Sweden shows the second highest milk consumption and France the highest cheese consumption, yet both have relatively low death rates from cardiovascular disease. In cheese xanthine oxidase is present in larger particle size and is most probably digested, not absorbed into the blood stream, thus precluding biological availability. The high saturated fatty acid content of milk and cheeses consumed in these countries and their relatively low cardiovascular death rates certainly speak against the theory that high saturated fat intake causes hypercholesterolemia followed by atherosclerotic coronary disease, leading to increased heart attack rates.

On a personal visit to East Africa it was learned that members of the Masai tribe who drink enormous amounts of milk (reportedly 7 liters daily) will "eat" the milk only in its curdled form. The same custom is observed by other East African tribes and also the Somali. Curdling produces large-sized particles, thus preventing absorption of xanthine oxidase.

Custom and tradition often determine the form of consumed liquid milk. Rural populations are more apt to boil their milk than residents of cities, where the wide availability and convenience of packaged milk favors consumption of the unboiled product.

The two known functions of xanthine oxidase are: 1. to oxidize xanthine to uric acid, and 2. to oxidize aldehydes to their respective acids. This enzyme has phoenix-like characteristics. By binding

its substrate to the enzyme complex it is possible for xanthine oxidase to exist in an inactive form which may then be reactivated by separating substrate from enzyme.[21] In both its active and reactivated forms it can oxidize aldehydic substances, whether they are situated in the arterial wall or in the heart muscle. This process could proceed in an extremely slow fashion, involving months of continued ingestion of milk and milk products. Homogenization would speed up this process. Ultimately, when sufficient aldehydes are oxidized in a specific anatomical location, a change from quantity to quality will occur, and the modified tissue structure will demand replacement of the depleted cell constituents. Theoretically, it should be at this stage that the fatty streak forms in the arterial wall. It is presumed that invasion of the fatty streak by cholesterol and fibrin will occur subsequently.

Discussion

Since the initiation of the atherosclerotic process is projected into youth, and still assuming a dietary origin of the atherosclerotic plaque, the search for a culprit should be limited to a food which is abundantly consumed in youth. Bovine milk offers itself as a natural possibility. The human alone of all mammals is subject to extensive, allegedly spontaneous atherosclerosis, and it is also the human who consumes milk after weaning. Bovine milk contains the enzyme xanthine oxidase which is not present in human milk. Homogenization of milk is one of the great changes in basic food composition which differentiates between the diet of our generation and that of our forefathers. This process was introduced because it imparted to the milk an allegedly improved taste and evinced a higher consumer acceptance as to stability and esthetic appearance.

One may well question if homogenization of milk represents a forward step in the progress of human nutrition. It is a procedure which foists unnaturally small particles on our digestive tract. Intestinal microvilli do not possess the biological properties for coping with these small particles, and, therefore, they pass unchallenged through a natural barrier which should defend the biological integrity of the organism. Micronized milk xanthine oxidase should also be able to penetrate the intestinal barrier and, like Reimann's microcrystals, pass into the blood stream with ultimate deposition on arterial walls. From a position in the coronary arteries penetration into the myocardium could be expected.

An enzyme is of no significance without a suitable substrate. A substrate for xanthine oxidase is provided by the aldehydic moiety of plasmalogen molecules which are so generously found in the outline of the arterial wall and in the myocardium. The enzyme would then, by oxidizing these aldehydes, create a histochemical change in the homeostasis of the site which could be overcome either by 1. creating more substrate (more aldehydes), or 2. initiating a healing process to form a biological scar. This healing process in the vessel wall would comprise the influx of fat cells which in conglomeration would form the fatty streak. As they so often cover foreign bodies (*e.g.*, stones in the gall bladder), so may cholesterol and other lipids attach themselves as products of body reaction to the formed scar, and the process of atherosclerosis might thereby progress.

It should be assumed that plasmalogens in other anatomical sites, such as the myelin sheath of nerve cells, might also be attacked by xanthine oxidase which is present through absorption from the intestinal canal or because of some mechanism of endogenous hyperproduction of this enzyme. If such an enzymatic oxidation would lead eventually to the development of the disease multiple sclerosis would have to be decided by more research. The biochemical possibility is, however, definitely present.

One is then postulating the existence of a new pathological entity which could be described as diseases of the plasmalogens. They would be initiated by plasmalogen deficiencies in specific histochemical locations engendered by enzymatic action. The specific disease entities would then develop as secondary reactions of the body to the abnormal deficiencies. These reactions would assume a different nature in different anatomical positions.

Elimination of active xanthine oxidase from the human diet would be much simpler to accomplish than the vague attempts at modification of cholesterol, saturated or unsaturated fatty acids, and carbohydrate intake which are now suggested to the public. Attempts at inhibiting the activity of xanthine oxidase have already been shown to be fruitful in reducing the need for nitroglycerin in angina pectoris and, by implication, in reducing the frequency and intensity of anginal attacks.

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Immune response to bovine xanthine oxidase in atherosclerotic patients

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KNOWLEDGE OF the etiology of the atherosclerotic process is virtually nonexistent, and because of this lack of knowledge the prevention and treatment of atherosclerosis is in a deplorable state. The problem is importunate; hence, potentially dangerous pseudosolutions based on expediency and temporizing are offered. A tenuous association of elevated serum cholesterol found in certain homogeneous population groups is made the fulcrum of a therapeutic approach by diet and drugs for the reduction of risk of the sequelae of atherosclerotic involvement. Grandiose schemes for serum cholesterol reduction in the prevention and treatment of atherosclerotic coronary heart disease have wide semiofficial sanction by government institutions and voluntary health organizations. Curiously enough, the same approach of serum cholesterol reduction is seldom advocated in the treatment of peripheral artery atherosclerosis, found mainly in the atherosclerotic brain syndrome and the ischemic lesions of the lower extremities affecting so many diabetic patients.[1]

A new theory of the genesis of the atherosclerotic process has been postulated.[2] In essence it claims that the dietary origin of this disease is initiated by the absorption through the intestinal wall of xanthine oxidase contained in bovine milk and that the enzyme is then carried by the lymph stream to the arterial vascular system and hence deposited by an insudative process into the vessel walls and the myocardium. In these ectopic sites xanthine oxidase alters the integrity of the cell membrane by oxidizing a phospholipid, plasmalogen. The resulting pathological processes have been named Plasmalogen Diseases, with specific pathological manifestations in different anatomical sites.

This theory has been attacked as nonviable because of a traditional belief that a large molecule such as xanthine oxidase (molecular weight 290,000) cannot be absorbed intact; and, consequently, presence of xanthine oxidase in ectopic locations[3] could not derive from bovine milk but could originate only from such endogenous sources as the intestine or the liver.

The present study is concerned with this problem of intestinal absorption of large protein molecules from dietary sources. Preliminary demonstration of such absorption has been forthcoming in many reports. One finds a summary of these findings in a publication by Davies.[4] Davies has also shown by specific hemagglutination of sensitized sheep blood cells that milk proteins were absorbed into the human bloodstream. In addition, he found that patients with ischemic heart disease had a higher, statistically significant, antibody level to milk proteins than patients without the symptoms of ischemic heart disease.

We attempted to duplicate Davies's findings in patients with manifest atherosclerotic diseases, such as coronary heart disease, atherothrombotic brain syndrome, and peripheral atherosclerosis (claudication and threatening gangrene). We expanded his study to include not only milk proteins but also specific antibodies to xanthine oxidase from bovine sources. It was hoped that results of this study would demonstrate conclusively that this enzyme is absorbed, as postulated, and that eventual antibodies to it could be traced through the various age groups, despite the fact that milk intake in

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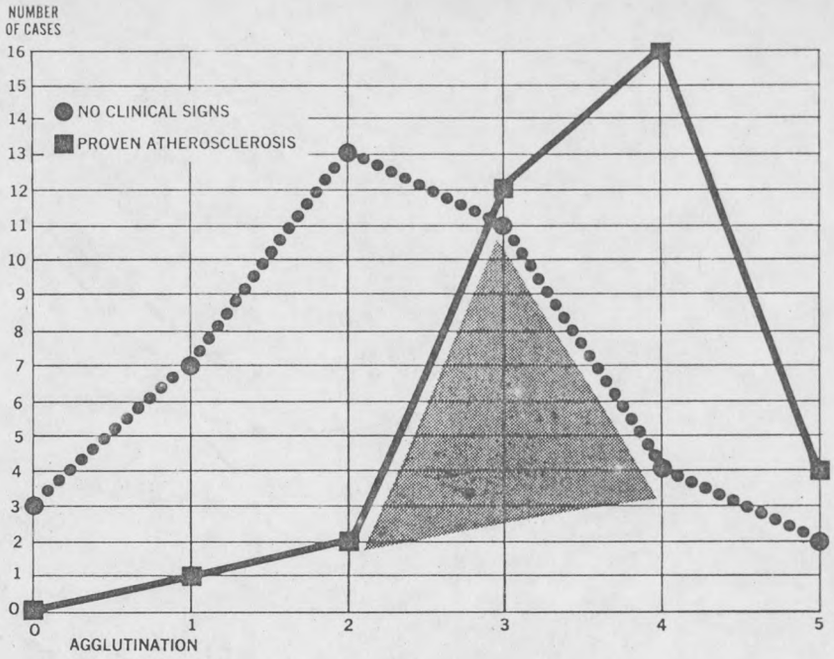

Figure 1 *Immunoassay of serum xanthine oxidase.*

adults is negligible compared with its greater consumption in youth.

Methods

Two separate antibody titrations were performed on each blood sample: a milk antibody test followed the method of Rees,[5] and xanthine oxidase antibodies were determined by an adaptation of the Boyden tanned erythrocyte technique.[6]

All solutions were prepared as described in Refs. 5 and 6, with one exception: the human sera were mixed with buffered saline in geometric 1:1 serial dilution instead of the neutral rabbit serum used by Rees. For the xanthine oxidase antibody test a 0.5% protein solution with sigma grade 2 xanthine oxidase* in 2.3 *M* ammonium sulfate solution was prepared by dilution (1:20) with 66.7% buffered saline and 33.3% of 0.66 *M* phosphate buffer (pH 7.4). Care was taken to ensure proper osmolarity for the sheep red blood cells.

Agglutination was measured on a scale of 0 to 5 by halves as an expression of each geometric dilution. A reading of 0 showed no agglutination, and a value of 5+ represented the highest antibody concentration measured.

Every investigated blood serum showed sequential agglutination. There was no pro-zone, and no serial dilution of the sera failed to show agglutination.

Controls

Control experiments were performed to rule out nonspecific agglutinations. A buffered saline solution that was added to the sensitized red sheep cells did not result in any agglutination. It was considered a negative control. A positive control was employed and consisted of unwashed, unsensitized sheep red blood cells diluted (1:60) with buffered saline to which was added an equal volume of undiluted, complement-inactivated serum.

Several sera were tested with both active xanthine oxidase antigen and heat-inactivated xanthine oxidase antigen. The antibody-antigen reaction was the same in both test groups. This finding supports the claim by Ultmann, Feigelson, and Harris[7] that the antigenic property of heat-inactivated xanthine oxidase is no less than that of active xanthine oxidase. Nevertheless, only further tests will prove if this important finding is reproducible in all specimens. Further, there must be a delineation of the limitations of the inactivation of xanthine oxidase before a possible screening of patients with inactivated xanthine oxidase can be initiated.

Patient material

Patients were selected at random by a registered nurse from an office practice of internal medicine. Under double-blind conditions the bloods were consecutively numbered, and none of the investigators was aware of the identity of any blood sample. When all tests were completed, the patients were divided into two groups by the senior author. (K. Oster) according to one criterion: presence or absence of clinical manifestations of atherosclerotic disease. These manifestations embraced all varieties of atherosclerosis, such as angina pectoris, myocardial infarction, serious cardiac arrhythmias, claudication, peripheral vascular disease with threatening gangrene, and atherosclerotic brain syndrome. Hypertensive and rheumatic valvular heart disease patients were considered as nonatherosclerotic for the purpose of the study.

*Sigma grade IV xanthine oxidase solution contains 112 mg protein/ml.

Results

The test group of 75 patient sera revealed varying titers of specific antibodies to bovine xanthine oxidase. Neither age nor sex exerted any apparent influence on the response. The study comprised 47 males and 28 females, whose ages ranged from 21 to 90 years. Surprisingly both the 21- and the 90-year old had high xanthine oxidase agglutination responses.

Figure 1 shows the frequency distribution of the various agglutination values found in the atherosclerotic and nonatherosclerotic patient groups. The abscissa indicates the various degrees of agglutination, 1+ through 5+. The ordinate lists the number of cases involved. The dotted line represents those patients with no detectable signs of atherosclerosis and the solid line the manifest atherosclerotics. Fractional values were rounded out to the next higher full value.

Two peaks are visible in the frequency distribution of the nonatherosclerotic and atherosclerotic groups. The nonatherosclerotics have mostly a 2+ agglutination reaction (13 cases) and a weak 3+ reaction (11 cases). The atherosclerotics show a peak agglutination of 4+ (16 cases) and 3+ (12 cases). The two groups overlap in a gray area between 2+ and 4+ agglutination. One may speculate that the group with no clinical manifestations of atherosclerosis may well have atherosclerotic lesions somewhere in their bodies which have not yet attained detectable status. On the other hand, those patients with known atherosclerosis who responded with low agglutination values may be the ones with poor general immunity. These were mostly people with thyroid deficiency.

Tables 1 and *2* list the individual test subjects and their agglutination responses (degree of antibody production) to milk proteins and to bovine xanthine oxidase. Scrutiny of a few individual cases reveals some interesting observations. A 47-year-old nonpatient, recruited as an apparently healthy person, had a 5+ xanthine oxidase agglutination response. When questioned about her milk history, she revealed that for many years the presence of a peptic ulcer had necessitated a diet high in cream and milk. Despite subsequent subtotal gastrectomy she had continued for two years her substantial intake of milk and cream.

Two young healthy Italian immigrants appeared for a required premarital blood test. Both had only 1+ agglutination. Their milk history revealed that the 24-year-old bride had been drinking only goat's milk most of her life, and the groom, a country boy, had been given only raw unrefrigerated cow's milk. It is known that raw untreated and unrefrigerated milk contains very little biologically available xanthine oxidase.[8]

Two natives of India were tested. Both were accustomed to drink boiled buffalo milk in their native land. The 28-year-old husband had a 1+ xanthine oxidase agglutination response, the 22-year old wife a 5+ agglutination. She suffers with chronic gastroenteritis and has been ingesting large quantities of homogenized cow's milk since living in this country.

In all likelihood absorption of foreign proteins through the damaged intestinal mucosa differs from absorption through the intact mucous membrane. One may well wonder how increase in penetrability of foreign proteins is enhanced by such commonly used drugs as acetyl salicylic acid, ethyl alcohol, emulsifiers, and detergents. It is hoped that these potential interactions of drugs and chemicals on our food utilization and absorption will be investigated and determined.

This study has led to one unavoidable conclusion. Bovine xanthine oxidase must have been absorbed through the intestinal wall, because specific antibodies to the enzyme were demonstrated in human sera. The major objection to the theory of the genesis of atherosclerotic lesions by absorbed xanthine oxidase has thereby been effectively countered.

Independent *t* tests were performed on the data for antibodies to both xanthine oxidase and to milk to determine whether significant differences existed between the groups. A total of 75 cases were tested for 75 degrees of freedom. The antibody-to-milk-protein test had an independent t value of 2.89, and so $p < 0.01$, which is very significant. Coincidently Davies obtained an independent t value of 2.85 in 50 cases with 48 degrees of freedom. The antibodies-to-xanthine-oxidase test had an independent t value of 4.86, and so $p < 0.001$, which is extremely significant.

The statistical significance of finding higher xanthine oxidase antibodies in test subjects with demonstrable atherosclerotic manifestations than in those with no clinical evidence of the disease should be submitted to further extensive testing. If confirmed by our ongoing investigation and by others, it could be developed into an important screening test. One might say that an individual with an antibody reaction of 3+ or higher would have atherosclerotic processes initiated by xanthine oxidase somewhere in his body. The location would have to be determined by more intensive examination. Such a test would be a vast improvement over the so often meaningless (because of its variability) serum cholesterol determination.

Table 1

Antibody response to milk proteins and bovine xanthine oxidase in blood serum of patients with no clinical manifestations of atherosclerosis

No.	Sex	Age	Antibody titer milk protein	Antibody titer xanthine oxidase	Diagnosis	Remarks
1	M	71	2	3	Rheum. H. D.[a]	Atrial fibrillation
2	M	59	3	3	Neuritis	
3	F	21	2	4		1 qt. of milk daily
4	F	60	0	1	Diab. mell.[b]	Hypertension
5	M	72	1	1		
6	F	76	1	1.5	Diab. mell.	Hypertension
7	F	24	1	1		Goat's milk
8	M	28	0	1		Raw cow's milk
9	F	24	1.5	2		
10	M	29	0	0		
11	M	59	1	2	Rheum. H. D.	
12	M	40	2	2		
13	M	71	2	2	Polycythemia	Venous thrombosis
14	M	63	2	2.5	Rheum. H. D.	Aortic stenosis
15	F	57	1	1	Neurosis	
16	M	63	3	2	Hypertension	
17	M	67	3	2	Hypertension	
18	M	48	3.5	5		
19	M	45	3	4	Hypertension	
20	M	58	2	3	Hypertension	
21	F	67	3	2	Rheum. H. D.	Atrial fibrillation
22	M	29	3	1		Boiled buffalo milk
23	F	22	3.5	5	Gastroenteritis	Boiled buffalo milk
24	F	57	1	0		
25	F	47	3	5		
26	M	47	3	3.5		
27	M	68	2	3		
28	M	57	1	0		Possible adrenal tumor. Boiled milk
29	M	38	1	2.5		
30	F	43	2	2		
31	M	68	2	1	Hypertension	
32	F	68	2	3		
33	F	67	2	2		
34	F	46	3	3		
35	F	66	3	2	Diab. mell.	
36	M	70	3	2	Diab. mell.	Hypertension
37	F	62	3	3		
38	F	74	2	3	Arthritis	
39	F	44	3	3		
40	M	29	2	2		
41	F	47	3	5	Peptic ulcer	Gastrectomy

[a]Rheum. H. D. = rheumatic heart disease.

[b]Diab. mell. = diabetes mellitus.

Table 2

Antibody response to milk proteins and bovine xanthine oxidase in blood serum of patients with atherosclerotic diseases

No.	Sex	Age	Antibody response to milk protein	Antibody response to xanthine oxidase	Diagnosis	Remarks
1	M	63	3	3	M. I.[a]	
2	M	39	3	4	M. I.	Angina pectoris. Coronary bypass
3	M	72	2	3.5	M. I.	
4	M	34	4	4	M. I.	
5	M	68	2.5	3.5	M. I.	
6	M	39	2.5	3	M. I.	
7	M	62	2	3.5	Angina pectoris	Coronary arteriography
8	F	60	3	3	M. I.	
9	M	60	2	4	M. I.	Angina pectoris. Psoriasis
10	M	50	2	4	M. I.	
11	M	61	2	3.5	M. I.	Diab. mell.
12	M	64	2	3	M. I.	Diab. mell.
13	M	59	1	3.5	Arrhythmia	
14	F	70	2	4	Claudication	
15	M	69	2	3	C. V. A.[b]	
16	M	60	2.5	4	M. I.	
17	M	62	2.5	4	M. I.	Diab. mell.
18	M	45	2	4	M. I.	
19	M	55	3	3	M. I.	Angina pectoris
20	F	72	2	3	Diab. mell.	Hypertension
21	F	37	2.5	3.5	Renal arter.[d]	Malignant hypertension. Gastrectomy
22	M	69	3	3.5	M. I.	Emphysema
23	M	71	2	2	Claudication	Diab. mell.
24	F	75	3	3	Brain ischemia	Hypertension. Pulmonary embolism
25	F	62	3.5	3	Brain ischemia	
26	F	55	3	1	M. I.	
27	M	63	3	3	M. I.	
28	F	75	3	4	Arter. H. D.[e]	Atrial fibrillation. Ileitis
29	M	54	3	2	M. I.	
30	M	57	2.5	3	Claudication	Diab. mell.
31	M	90	3	5	C. V. A.	Arteriosclerotic H. D. Pacemaker
32	F	72	3	5	Arter. H. D.	Atrial fibrillation
33	M	60	2	3	M. I.	
34	M	64	4	4.5	M. I.	Angina pectoris

[a]M. I. = myocardial infarction.
[b]C. V. A. = cerebrovascular accident.
[c]Diab. mell. = diabetes mellitus.
[d]Renal arter. = renal arteriosclerosis.
[e]Arter. H. D. = arteriosclerotic heart disease.

M-25
F-9

34 | 2068
60.82

3.53 - age

It is not known if a high level of antibody to xanthine oxidase is beneficial or harmful to the individual. We have opted for the former position. Ultmann et al.[7] have found that antibodies to xanthine oxidase inhibit the activity of the enzyme up to 70% because of noncompetitive antibody-antigen binding. One might then assume that antibodies to bovine xanthine oxidase are the body's defense mechanism against persistent ectopically deposited bovine xanthine oxidase. The argument that the ectopically found enzyme originates from the liver or the intestine is invalid according to our study, in which we have found close correlation between the antibody level and increased bovine milk intake. Additionally, the differences between milk antibody and xanthine oxidase antibody responses by the same test individual would not have occurred, had the enzyme originated from autogenous human sources.

One of the most unexpected findings in this study was the observation that patients up to 90 years of age still have measurable antibodies to bovine xanthine oxidase in their sera. This demonstration together with the published finding[3] of active xanthine oxidase in atherosclerotic plaques and myocardial tissue in elderly patients should serve as a major stepping-stone to the experimental proof of the origin of the atherosclerotic lesion, that of bovine xanthine oxidase attacking plasmalogen in the cell membrane, a pathological process which has been termed plasmalogen disease.

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A new culprit in atherosclerotic disease

Enzyme may affect the arterial wall

Dr. Oster exhibits device used in homogenization of milk.

The search for the pathogenesis of atherosclerosis at times takes on the aspects of the alchemist's search for the philosopher's stone. Many have an idea about it, some claim to have found it, but no one has come up with a proveable answer.

To the growing list of theories about the initial lesion of atherosclerosis must be added that of a German-born cardiologist who believes that it can be traced to the action of an enzyme found in cow's milk on the intima of the arterial wall. He notes that atherosclerosis has its origin in youth, when there is high consumption of cow's milk.

According to Dr. Kurt A. Oster, chief of cardiology at Park City Hospital in Bridgeport, Conn., and medical director of McKesson Laboratories, the enzyme, xanthine oxidase, oxidizes the aldehydes of a group of chemicals known as plasmalogens, which are an important component of the arterial wall. It is at the site of such tissue destruction that cholesterol deposition begins.

The plasmalogens are chemicals found in a variety of organs, Dr. Oster explains. They abound not only in the arterial wall but in muscle tissue and the myelin sheath of nerve fibers. They are absent, however, from the liver and mucous membranes of the small intestine.

These latter tissues, however, do contain xanthine oxidase, found abundantly in cow's milk but not in human milk. In one study, Dr. Oster found that when xanthine oxidase was reacted chemically with plasmalogen, the resulting compound was oxidized and destroyed; this explains the absence of plasmalogen from the liver and small intestine.

Depletion of plasmalogen in organ tissues, where its function is vital, could occur if xanthine oxidase were overabundant, or if its activity could be simulated, or if it were mobilized from its storage position in the liver and small intestine. It could then exercise its aldehyde-oxidizing power in areas where these aldehydic substances are vital for cell equilibrium or biological functioning.

"If enough of these substances were oxidized by excess xanthine oxidase, you'd be left with a pathologic deficiency that the body might try to rectify," the Connecticut cardiologist explains. "This might take the form of lipid infiltration, as witness the fatty streak in the arterial wall, or eventual tissue necrosis, as seen in the myocardium."

But how does xanthine oxidase enter the bloodstream? There is no evidence to suggest that it is mobilized from its natural sites in the liver and small intestine. The other possibility is ingestion and absorption. One of the basic laws of pharmacology holds that particle size determines rate of absorption into the body, and that when particles are micronized, it is easier for them to get into the bloodstream from the intestine.

Xanthine oxidase, a part of the microsomal particle, is located in the fat globules of milk. Since 1938, Dr. Oster points out, milk in the U.S. has been homogenized routinely. In the process, the diameter of the fat globules is reduced to less than a third of their natural size. These extremely small particles can then be absorbed through the intestinal mucous membranes, to be deposited eventually on the arterial wall or in the heart muscle.

"Nutritionists think you take something out of a can, count the calories, put it in your mouth, and that's it. But if you're a pharmacologist, you know that if you put a drug in large crystalline form, it is absorbed differently than when in a small crystalline form," says Dr. Oster, who taught pharmacology for 15 years.

If the concept of plasmalogen disease of dietary origin is to stand up to scrutiny, three things have to be shown, he says: that xanthine oxidase is present in food, that it occurs in the pathological lesion and not in normal tissue, and that the lesion is reproducible in other species.

The first two points have been proved to Dr. Oster's satisfaction. However, he points out, because animals probably do not absorb food

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the way humans do, it may well be impossible to reproduce the lesion. And since experimental administration of the enzyme to humans is impracticable, he says, one is forced back on epidemiology, demonstrating a correlation between the consumption of homogenized milk in a population and the rate of atherosclerotic disease.

In a study of the milk-drinking habits of ten countries, using information acquired from the various national dairy industries, Dr. Oster found a high correlation between the death rate from heart disease and the consumption of homogenized milk (see chart). At the top of the list are the Finns, who drink more milk than any other group for whom Dr. Oster obtained data (593 pounds per person annually) and who have the highest death rate from atherosclerosis-connected disease (244.7 per 100,000 in 1967). The Finnish dairy industry estimates that a third of the milk consumed in that country is homogenized.

Americans drink about 273 pounds of milk per capita and have 211.8 deaths per 100,000. Almost all U.S. milk is homogenized. Although the Swiss drink more milk than the Americans, they have comparatively low incidence of heart disease. This, Dr. Oster believes, is because they normally heat their milk before drinking it, which inactivates the xanthine oxidase. The same is true in France, Sweden, and Italy.

The Connecticut cardiologist notes an apparent paradox. The French, who are Olympic cheese eaters, rank almost at the bottom of the heart disease mortality scale. The explanation, he says, is that xanthine oxidase exists in large particles in cheese and therefore is digested and excreted rather than being absorbed into the bloodstream.

"Considering the high saturated fatty acid content of cheese, this finding should certainly prove something to the cholesterol people," declares Dr. Oster. As additional evidence of his theory, he points to the Masai tribe of East Africa, among whom each person consumes some 7 liters of milk a day, making their diet about 60% saturated fats. Yet they have no atherosclerosis or hypercholesteremia. Dr. Oster attributes this to the fact that they drink curdled milk, which comes in large particles and thus provides no biologically available xanthine oxidase. Similar phenomena are observed in countries with high consumption of yoghurt and buttermilk, such as Albania, he adds.

Certain investigators insist that the Masai, a group much studied by epidemiologists, have a genetic trait that allows them to metabolize cholesterol faster than other people. The fact remains, however, that the relative absence of atherosclerosis in the Masai runs counter to the hypercholesteremic theory of heart disease, the Connecticut cardiologist notes.

Because there is no way to observe or measure the atherosclerotic process, this kind of epidemiologic information is the only tool available to work with, Dr. Oster states.

There is no reason why manufacturers in this country could not preboil their milk, Dr. Oster points out. Although some vitamins would be lost, the amount would be negligible. However, he says, the U.S. dairy industry has shown no interest in the idea. Meanwhile, Dr. Oster has himself taken out a patent on "XO-free milk" in Australia and New Zealand, where interest has been expressed in his theory of the genesis of atherosclerotic disease.

Dr. Oster suspects that plasmalogen disease has various manifestations, and he has been searching for a xanthine oxidase inhibitor. For his patients with hyperuricemia and angina pectoris, he first came up with allopurinol, which was effective, he says, but eventually proved toxic to the liver.

He is now working with large doses of folic acid and claims to have achieved success in diminishing the frequency of anginal attacks. He expects further studies with the vitamin to provide confirmation of his thesis. "When two different chemicals that have one common target effect also produce the same clinical effect, then we've taken a giant step forward," he declares.

Although his theory has been published in the medical literature, there has been little acceptance by the profession. However, he remains undiscouraged, and continues to pursue his in vitro studies.

"I have to admit the possibility that I'm wrong, but so far no one has been able to disprove me," Dr. Oster says, smiling. ■

ATHEROSCLEROSIS DEATH RATE AND FLUID MILK CONSUMPTION

Country	Death rate per 100,000 (1967)	Milk intake (pounds per person)	Homogenized	Preboiled
Finland	244.7	593	About 33%	No
United States	211.8	273	Almost all	No
Australia	204.6	304	15%	No
Canada	187.4	288	Partly	No
United Kingdom	140.9	350	About 7.5%	No
The Netherlands	106.9	337	Infrequently	—
West Germany	102.3	213	Partly	—
Austria	88.6	327	Occasionally	—
Italy	78.9	137	12.5%	Yes
Switzerland	75.9	370	Small quantity	Yes
Sweden	74.7	374	—	Yes
France	41.7	230	Negligible	Yes
Japan	39.1	48	Occasionally	—

Chart ties milk-drinking to deaths from atherosclerotic disease.

PLASMALOGEN DISEASES: A NEW CONCEPT OF THE ETIOLOGY OF THE ATHEROSCLEROTIC PROCESS

Kurt A. Oster, M.D.*

A new disease entity called Plasmalogen Disease is proposed. It originates from the oxidation of the plasmal aldehydes of plasmalogen by ectopic xanthine oxidase. This enzyme may be absorbed by ingestion, especially of the micro-sized droplets found in homogenized bovine milk. It is found in atherosclerotic plaques in which the amount of normally present plasmalogen is greatly diminished. Similar observations can also be made in the myocardium. Fatty streaks, the precursors of atherosclerosis, are early manifestations of plasmalogen disease. A comparison of death rates from myocardial infarction with the consumption of dairy products in various nations shows no correlation whatsoever with the intake of saturated fatty acid but a very close fit with the intake of biologically available xanthine oxidase. A simple method for the primary prevention of atherosclerosis in youth would be the creation of a biologically available xanthine oxidase-free milk. Interim steps recommended would be the cessation of homogenization and/or preheating of milk by the consumer.

In 1958 the World Health Organization introduced a system of classifying atherosclerotic lesions, starting the sequential process with those lesions, already found in youth, designated as "fatty streaks."[1] Placing the initiation of the atherosclerotic process in the period of adolescence has met increasing acceptance. Since these changes are evident at such an early age, it is difficult to reconcile the genesis of the fatty streak with such frequently mentioned risk factors as hypertension, obesity, cigarette smoking, lack of physical activity, and emotional stress-provoking situations. The findings of Enos *et al*[2] that 12% of American soldiers killed in the Korean War exhibited 50% occlusion of the coronary lumen, and 5% had 90% occlusion, speak against the theory of hypercholesterolemia and electrocardiographic abnormalities, which conditions were not present in these young men. Similar findings were reported in a Chilean study.[3]

Even if one does not equate the presence of this excessive amount of coronary occlusion with later manifestations of ischemic heart disease and eventual myocardial infarction, one must admit that a severe degree of atherosclerosis can exist in young people. Are we not then justified in questioning the cause of these fatty streaks in the adolescent who exhibits no trace of hyperlipidemia? Conversely, the temptation is great to doubt the vigorously promoted theory that hyperlipidemia is the cause of these lesions and that primary prevention directed to reducing the cholesterol level in blood serum by dietary manipulation may be based on false premises.

The hyperlipidemia theory of atherosclerosis has been in existence for more than fifty years. Because it attributed the origin of atherosclerosis to an increase of various fatty substances in the plasma, it has proposed prevention of the condition by such dietary changes as low cholesterol and normal fat, low fat, normal cholesterol and lower saturated fats, and increase in polyunsaturated fats. This has spawned a deluge of animal experiments designed to mimic human pathology, and much of the work has caused confusion by creating conditions of unreality and by producing strange diseases which do not fully simulate human atherosclerosis. If we free ourselves from this hyperlipidemia theory, we might then postulate that the etiology of atherosclerosis lies not necessarily in an overabundance of such fatty substances as cholesterol, triglycerides, or lipoproteins, but rather in a deficiency of defined chemicals localized in the arterial intima, as the first step, and the infiltration

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by cholesterol esterified with unsaturated oleic acid[4] in the depleted area, as the sequela. This infiltration might be described as the body's repair mechanism to the original noxa.

The various plasmalogens, chemicals normally found in the arterial intima and also in cardiac muscle tissue, fulfill admirably the requirements of this deficiency theory of the onset of atherosclerosis. For the last thirty years the author has marvelled at the selectivity of the histochemical localization of the plasmalogens. Their eventual biologic significance in human metabolism has been studied in both animal and human experimentation.

Since plasmalogens are unfamiliar to many clinicians, the following may serve as a brief introduction to their chemical structure. Plasmalogens are polar substances related to lecithin, in the older terminology, or phosphatidyl choline, in the newer nomenclature. One of the plasmalogens is an enol ether of either palmitic or stearic aldehyde attached to a lysoplasmalogen: $CH_2OCH{=}CHR^1$

$$\begin{array}{l} RCOOCH \quad O \\ \qquad\quad \| \\ \qquad CH_2OPOCH_2CH_2N\,(CH_3)_3 \\ \qquad\quad | \\ \qquad\quad O \end{array}$$

R^1 = Fatty aldehyde; R = Unsaturated fatty acid

Cleavage of the aldehyde moiety from the lysoplasmalogen may be effected in histochemical examination by $HgCl_2$ or other heavy metal salts, by weak acids, or, biologically, by the enzymes phospholipase A and vinyletherase, which are present in many tissues. Plasmalogen aldehydes are called plasmal. Choline plasmalogens are abundantly present in heart muscle, whereas in the myelin sheath of the central nervous system colamine or serine plasmalogens prevail. There are also many species variations in plasmalogen chemistry. Nevertheless, it has been stated that *up to 30% of the phospholipids found in heart muscle are plasmalogens,* especially those situated in the cell membrane. One wonders why so little is known about the biological significance of these substances which have such wide distribution in vital tissues.

Anatomical location of the plasmalogens has been thoroughly reviewed by Oster.[5] In addition to the arterial intima and the myelin sheath of nerve tissue, plasmalogens are found in certain portions of the kidney, mucous membranes of the large intestine, the adrenal cortex, and many other distinct organs. It is of great interest that certain metabolically active organs are devoid of plasmalogens, namely, the liver and mucous membranes of the small intestine. The absence of plasmalogen from these anatomical sites was ascribed by Oster and Mulinos[6] to the activity of the enzyme *xanthine oxidase, which is present in abundance in those locations where plasmalogen is absent.* It was shown at that time that when plasmalogen was split into aldehyde and lysoplasmalogen by the action of dilute HCl, the resulting aldehydes could be oxidized by xanthine oxidase derived from milk

Plasmalogens were shown to be target substances for the action of male and female sex hormones. Oster[7] proved that the distribution of these chemicals in the female rat kidney was governed by the phases of the estrous cycle. It was demonstrated that the disappearance and reappearance of plasmalogens in the rat kidney could be duplicated in the castrated animal by the administration of male or female hormones. These changes, should they be shown to occur also in the human organism, might possibly explain the lower incidence of myocardial infarction in women of child-bearing age than in men of equivalent years.

Oster and Hope-Ross[8] performed a histochemical examination of cardiac muscle from a case of fatal myocardial infarction and found that plasmalogen had disappeared from the infarcted area less than two hours after the onset of pain. There was no necrosis or other significant tissue changes of the affected heart muscle. In effect, plasmalogen leaked out of the infarcted region *before* any other demonstrable changes occurred. Surely this substantiates the theory that plasmalogens are associated with

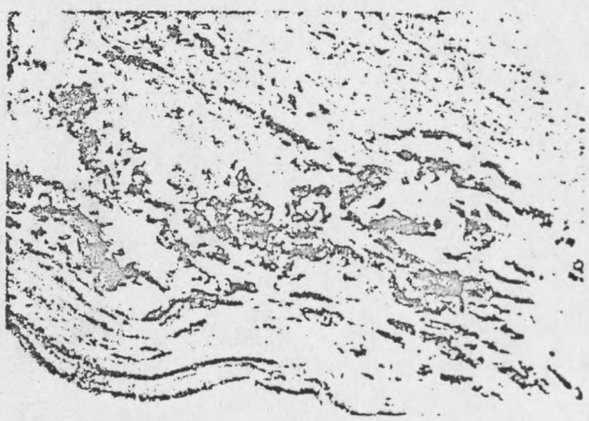

Figure 1. Aorta of a 22 year old apparently healthy drowning victim. Fuchsin sulfurous acid plasmal reaction x 480. Plasmalogen has disappeared from behind the intact intima. The darkish material infiltrating the area where plasmalogen has disappeared most likely consists of cholesterol esterified with monounsaturated oleic acid.

myocardial damage.

Similar absence of plasmalogen was found in the aorta of a 22 year old drowning victim suffering from extensive atherosclerotic changes of this blood vessel (See Fig. 1). Buddecke and Andresen[9] also have shown that increased plasmalogen loss from the aortic wall corresponds to an increase in atherosclerosis.

Making use of the described observation that xanthine oxidase destroys the aldehydes liberated from plasmalogen,[6] Oster attempted to restore depleted plasmalogens to the myocardium by inhibiting xanthine oxidase action with a known xanthine oxidase inhibitor, allopurinol.[10] This clinical effort, which was undertaken in sixteen patients suffering with angina pectoris, met with gratifying results. A dose-effect relationship could be established between the quantity of allopurinol and the reduction of nitroglycerin needs. It was then postulated for the first time that angina pectoris and myocardial infarction may be *plasmalogen diseases* and that manifestations of plasmalogen depletion might also be found in other histochemical sites, *e.g.*, in the myelin sheath in multiple sclerosis.[11]

Such a plasmalogen depletion could occur if there existed an overabundance of xanthine oxidase, or if the activity of this enzyme could be stimulated, or if it were mobilized from its storage position in the liver and small intestine,[6] thereupon to exert its aldehyde-oxidizing power where the presence of these aldehydic substances is vital and necessary for the persistence of cell equilibrium or biological functioning. Milch's studies have proven the presence of aldehydes to be essential for the maintenance of elasticity in the arterial wall.[12] Also, Oster has postulated that tissue aldehydes may serve as receptor substances for certain amines and other pharmacologically active radicals.[13] If enough of these biologically essential aldehydic substances should be oxidized by excess xanthine oxidase, a pathological deficiency would result which the body might try to rectify. Such corrective steps may take the form of lipid infiltration,[4] as witness the fatty streak in the arterial wall, or eventual tissue necrosis, as seen in the myocardium.

In the following the author will show how xanthine oxidase may enter the blood stream by ingestion. The only food containing a significant amount of this enzyme is bovine milk. The milk industry in this country pasteurizes milk at 170°F. with a fifteen second holding period, and this practice leaves about 42% of the xanthine oxidase in its active state.[14]

One of the basic tenets of pharmacology states that particle size determines rate of absorption into the body, and micronization of particles facilitates their entry into the blood stream from the intestine.[15] Xanthine oxidase in milk, a part of the microsomal particle, is situated in the fat globules, and for the past thirty years the size of these fat droplets has been altered, micronized, "homogenized." Homogenization of milk became a routine practice in the United States about 1938.

"The globules of normal cow milk vary in size from about 0.1μ to about 15μ in diameter, averaging about 3.5μ with 80% in the 2.0 to 5.0μ range ... When milk is homogenized, the fat globules are broken up and reduced in size from an average diameter of 3.5μ to an average of about 1μ. This increases the number of globules some one hundred times and expands the fat globule surface between six and seven times."[16]

Reimann in his studies of microcrystal absorption has proved that completely insoluble crystals of non-absorbable material do, when present as microcrystals, penetrate through the intestinal mucosa and are transported through the microvilli into the venous and the arterial blood, via the lymph system, and are found deposited in several body locations, including the myocardium.[17]

It is then the smallness of the particle size which creates a completely new aspect of pharmacological behavior. Microcrystals attain blood levels not usually accomplished by the regular crystal size of such drugs as griseofulvin.[18] Thus, homogenization of milk, creating micronized fat globules, will enhance the absorptive potential of xanthine oxidase through the intestinal mucosa whence it eventually reaches the blood stream to become biologically available.

If Koch's three postulates for establishing the presence of an organism causing a specific infection were to be applied to the concept of plasmalogen disease caused by xanthine oxidase of dietary origin, one would have to prove its presence in the food, its presence in the pathological lesion and its absence from normal tissue, and, finally, the reproducibility of the lesion in other species. The first two points have been investigated by Ross, Malts and Oster,[19] and by Ross, Ptaczinski and Oster[20] respectively. Their examination of milk products obtained from a dairy and grocery shelves demonstrated the presence of xanthine oxidase, especially in homogenized milk and even the so-called 99% fat-free milk, also homogenized. Exam-

ination of autopsy material from two men, 54 and 74 years old, clearly demonstrated the presence of xanthine oxidase in the atherosclerotic plaque and its almost complete absence from adjacent normal tissue. Similar findings were obtained from cardiac muscle tissue.

Xanthine oxidase, then, satisfies two of Koch's three postulates. To produce atherosclerosis in experimental animals by the ingestion of xanthine oxidase one must assume that the animals have the same intestinal absorptive capabilities as do humans, and this is most unlikely. The administration of xanthine oxidase to humans on an experimental basis is impracticable, since tissue results can be studied only in autopsy material.

However, Koch's third postulate can be satisfied retrospectively by studying the milk consumption of large population groups, comparing the intake of biologically active xanthine oxidase-containing milk products with the published frequency of death from arteriosclerotic and degenerative heart disease. Table I is such a comparison.

TABLE I

Country	1967 Death Rate	Pounds Per Person Fluid Milk Intake	Pounds Per Person Butter	Pounds Per Person Cheese	Relative Standing M	Relative Standing B	Relative Standing C	Homogenized	Pre-Boiled Frequently
1. Finland	244.7	593	35.	7.3	1	1	12	1/3	No
2. United States	211.8	273	4.7	10.6	9	11	7	almost all	No
3. Australia	204.6	304	22.9	7.8	7	2	11	not general practice	
4. Canada	187.4	288	16.2	9.0	8	8	9	partially	No
5. United Kingdom	140.9	350	19.7	11.0	4	4	6	about 7½%	No
6. Netherlands	106.9	337	5.7	19.5	5	10	4	infrequently	
7. F.R. of Germany	102.3	213	18.7	9.3	11	5	8	partially homogenized	
8. Austria	88.6	327	13.2	8.4	6	9	10	occasionally homogenized	
9. Sweden	74.7	374	16.3	18.3	2	7	5		Yes
10. Italy	78.9	137	4.0	19.9	6	12	3	12.5%	Yes
11. Switzerland	75.9	370	16.4	22.1	3	6	2	small quantity	Yes
12. France	41.7	230	19.9	28.8	10	3	1	negligible	Yes
13. Japan	39.1	48	?	?				occasionally	

Comparison of Death Rates per 100,000 Population from Arteriosclerotic and Degenerative Heart Disease with Consumption of Milk and Dairy Products.

Death rates (45-54 years old). Source: World Health Statistics Annual, World Health, Aug.-Sept. issue p. 11, 1970.

Consumption of fluid milk and cream, butter, and cheese in selected foreign countries, 1968. Source: Butz, W.T., How Americans Use their Dairy Foods, National Dairy Council, Chicago, Ill., p.15, 1970. Data on homogenization and customs obtained from the respective national dairy institutes. The cooperation of those in charge is gratefully acknowledged.

We find that Finland has both the highest death rate from heart disease and the highest rate of milk consumption. The United States, with the second highest death rate, has a relatively low milk intake. However, most milk in this country is homogenized, and homogenization multiplies the biological availability of xanthine oxidase by at least 3.5, making it 3.5 times as great as that found in non-homogenized milk. U.S. milk consumption can thus be regarded as 273 lbs. x 3.5 (homogenization factor), or the equivalent of 955.5 lbs. per person of pasteurized unhomogenized milk. The Finns surpass this figure, for according to data received about one-third of their milk is homogenized, giving them an approximate equivalent consumption of 1086 lbs. of xanthine oxidase-active milk.

The table shows that Australia and Canada present high death rates from cardiovascular disease yet occupy seventh and eighth places respectively in their fluid milk consumption. Here the questions of homogenization and preboiling are not completely resolved from the information received from their national dairy institutes. However, those countries which report a low mortality from cardiovascular disease, less than one-half that of Finland and the United States, frequently have the custom of preboiling milk before consumption, thereby biologically inactivating its contained xanthine oxidase. Sweden shows the second highest milk consumption and France the highest cheese consumption, yet both have relatively low death rates from cardiovascular disease. In cheese xanthine oxidase is present in larger particle size and is most probably digested, not absorbed into the blood stream, thus precluding biological availability. The high saturated fatty acid content of milk and cheeses consumed in these countries and their relatively low cardiovascular death rates certainly speak against the theory that high saturated fat intake causes hypercholesterolemia followed by atherosclerotic coronary disease, leading to increased heart attack rates.

On a personal visit to East Africa it was learned that members of the Masai tribe who drink enormous amounts of milk (reportedly 7 liters daily) will "eat" the milk only in its curdled form. The same custom is observed by other East African tribes and also the Somali. Curdling produces large-sized particles, thus preventing absorption of xanthine oxidase.

Custom and tradition often determine the form of consumed liquid milk. Rural populations are more apt to boil their milk than residents of cities, where the wide availability and convenience of packaged milk favors consumption of the unboiled product.

The two known functions of xanthine oxidase are: 1. to oxidize xanthine to uric acid, and 2. to oxidize aldehydes to their respective acids. This enzyme has phoenix-like characteristics. By binding

its substrate to the enzyme complex it is possible for xanthine oxidase to exist in an inactive form which may then be reactivated by separating substrate from enzyme.[21] In both its active and reactivated forms it can oxidize aldehydic substances, whether they are situated in the arterial wall or in the heart muscle. This process could proceed in an extremely slow fashion, involving months of continued ingestion of milk and milk products. Homogenization would speed up this process. Ultimately, when sufficient aldehydes are oxidized in a specific anatomical location, a change from quantity to quality will occur, and the modified tissue structure will demand replacement of the depleted cell constituents. Theoretically, it should be at this stage that the fatty streak forms in the arterial wall. It is presumed that invasion of the fatty streak by cholesterol and fibrin will occur subsequently.

Discussion

Since the initiation of the atherosclerotic process is projected into youth, and still assuming a dietary origin of the atherosclerotic plaque, the search for a culprit should be limited to a food which is abundantly consumed in youth. Bovine milk offers itself as a natural possibility. The human alone of all mammals is subject to extensive, allegedly spontaneous atherosclerosis, and it is also the human who consumes milk after weaning. Bovine milk contains the enzyme xanthine oxidase which is not present in human milk. Homogenization of milk is one of the great changes in basic food composition which differentiates between the diet of our generation and that of our forefathers. This process was introduced because it imparted to the milk an allegedly improved taste and evinced a higher consumer acceptance as to stability and esthetic appearance.

One may well question if homogenization of milk represents a forward step in the progress of human nutrition. It is a procedure which foists unnaturally small particles on our digestive tract. Intestinal microvilli do not possess the biological properties for coping with these small particles, and, therefore, they pass unchallenged through a natural barrier which should defend the biological integrity of the organism. Micronized milk xanthine oxidase should also be able to penetrate the intestinal barrier and, like Reimann's microcrystals, pass into the blood stream with ultimate deposition on arterial walls. From a position in the coronary arteries penetration into the myocardium could be expected.

An enzyme is of no significance without a suitable substrate. A substrate for xanthine oxidase is provided by the aldehydic moiety of plasmalogen molecules which are so generously found in the outline of the arterial wall and in the myocardium. The enzyme would then, by oxidizing these aldehydes, create a histochemical change in the homeostasis of the site which could be overcome either by 1. creating more substrate (more aldehydes), or 2. initiating a healing process to form a biological scar. This healing process in the vessel wall would comprise the influx of fat cells which in conglomeration would form the fatty streak. As they so often cover foreign bodies (*e.g.*, stones in the gall bladder), so may cholesterol and other lipids attach themselves as products of body reaction to the formed scar, and the process of atherosclerosis might thereby progress.

It should be assumed that plasmalogens in other anatomical sites, such as the myelin sheath of nerve cells, might also be attacked by xanthine oxidase which is present through absorption from the intestinal canal or because of some mechanism of endogenous hyperproduction of this enzyme. If such an enzymatic oxidation would lead eventually to the development of the disease multiple sclerosis would have to be decided by more research. The biochemical possibility is, however, definitely present.

One is then postulating the existence of a new pathological entity which could be described as diseases of the plasmalogens. They would be initiated by plasmalogen deficiencies in specific histochemical locations engendered by enzymatic action. The specific disease entities would then develop as secondary reactions of the body to the abnormal deficiencies. These reactions would assume a different nature in different anatomical positions.

Elimination of active xanthine oxidase from the human diet would be much simpler to accomplish than the vague attempts at modification of cholesterol, saturated or unsaturated fatty acids, and carbohydrate intake which are now suggested to the public. Attempts at inhibiting the activity of xanthine oxidase have already been shown to be fruitful in reducing the need for nitroglycerin in angina pectoris and, by implication, in reducing the frequency and intensity of anginal attacks.

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ROLE OF PLASMALOGEN IN HEART DISEASES

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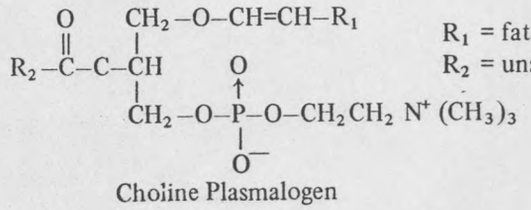
Although a majority of investigators accept the concept of a dietary origin for atherosclerosis and heart disease, there is a wide disagreement as to the nature of the dietary source responsible for the genesis of the atherosclerotic plaque. The controversy between promoters of the saturated fatty acid theory of origin and those who accuse simple carbohydrates is secondary to the almost unanimous indictment of high blood cholesterol levels as a major cause of vascular-induced heart muscle pathology. The recommended dietary changes deriving their rationale from the hypercholesterolemia concept are largely uninspiring, unrealistic, and impractical for the population at large. An increasing need is felt for new ideas to elucidate the origin of the chemical processes leading to both atherosclerosis and heart disease, since present theories are disappointingly unproductive.

This study attempts such a different approach. Generally recognized laws of biology, pharmacology, and epidemiology, together with histochemical observations, have been applied to the metabolism of a phospholipid, plasmalogen, situated strategically in the cell membrane, that critical area which is involved in the electrophysiological phenomena of heart action and is the target of so many drugs used in treating heart disease. The following data are presently available to support the postulated new concept of *plasmalogen disease.*

PLASMALOGENS

Chemistry

Rapport and Franzl (22) described the chemical formula of plasmalogen as a vinyl ether of glycerophosphocholine with one of the hydroxyl groups esterified by an unsaturated fatty acid. Choline plasmalogens are prevalent in heart muscle, comprising 20–30% of all the phospholipids found in that organ. Ethanolamine plasmalogen, on the other hand, is prevalent in the myelin of nerve tissue.



Choline Plasmalogen

Two enzymes are involved in the metabolic degradation of plasmalogen: phospholipase A, an algesic substance found in heart muscle and also in bee and snake venoms; and vinyl etherase, described in 1965 (31). The pharmacological qualities of the latter enzyme are not yet well known. Magnesium ions enhance the cleavage of ethanolamine plasmalogen (2). Phospholipase A hydrolyzes unsaturated fatty acids, and vinyl etherase hydrolysis results in plasmals, aldehydic compounds, usually stearal or palmital. The metabolism of plasmalogens is described in figure 1.

Histochemistry

Plasmalogens are found in specific locations in many organs. They are abundantly present in muscle tissue and in the myelin sheath of nerve fibers (15), but are not present in the liver or in the mucous membranes of the small intestine.

Pharmacology

Oster (18, 19, 21) has demonstrated that plasmal aldehydes may act as acceptor substances for many active drugs that contain free $-NH_2$ or =S-H

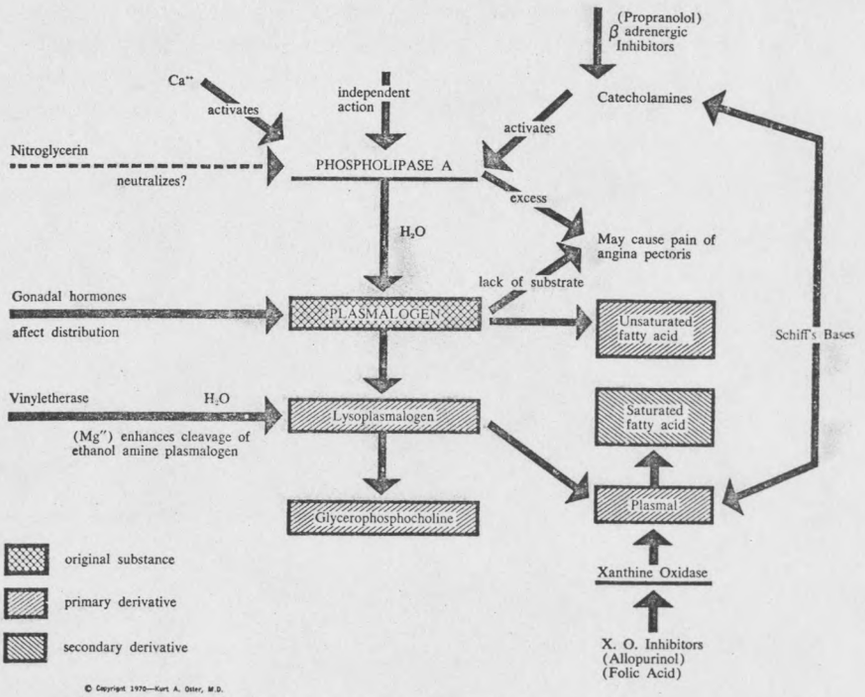


Fig. 1 Proposed pathway of plasmalogen metabolism.

groups. Table I lists some reacting and non-reacting drugs and chemical compounds. Some compounds having cardiac rhythm-controlling effects are found among the combining drugs.

Endocrinology

Oster (13, 14, 20) found that plasmalogen distribution in the intercorticomedullary zone of the rat kidney was influenced by sex hormones, the male hormones effecting a deposition of plasmalogen while estrogens and progesterone exert a plasmalogen-depleting effect. Waxing and waning of plasmalogen in the intercorticomedullary zone of the female rat kidney followed the pattern of the estrous cycle. It was not established which powerful male-like hormone in the rat hormonal equipment possessed the necessary strength to overcome the estrogen and progesterone effect.

Pathology

Miller *et al.* (11) described a diminution of plasmalogen in the atherosclerotic lesion. Oster and Hope-Ross (17) observed the disappearance of plasmalogen from heart muscle without concomitant cell necrosis, in a case of fatal myocardial infarction. On examining the aortas of juvenile drowning victims, Oster (16a) noted that the extensive fatty streaks present did not contain plasmalogen.

XANTHINE OXIDASE

Chemistry

Xanthine oxidase, an enzyme found in certain mammalian tissues, catalyzes the oxidation of purines, aldehydes, and other compounds. Xanthine is oxidized

Table I

Drugs and Chemicals as Reactants with Plasmal Aldehydes

Combining	Noncombining
Barbituric acid	Acetanilide
Thiobarbituric acid	Nitroaniline
β-Phenylethylamine	2-Aminopyridine
Cystein HCl	Benzylamine
Paraaminobenzoic acid	*d,l*,α-Phenylethylamine
Hydralazine HCl	Tyramine HCl
Rhodanine	Amphetamine sulfate
Aminophenol	Ephedrine sulfate
Toluidine	Histamine phosphate
Naphthylamine	Urea
Benzidine	Amino Acids

to uric acid, aldehydes to the corresponding acids. It was found that plasmal aldehydes, when liberated from their plasmalogen bondage by the action of HCl, were oxidized by xanthine oxidase to stearic and palmitic acids (18). Xanthine oxidase has been described as a metallo-flavo-protein, containing molybdenum and iron bound firmly to the apoprotein. Schardinger (27) reported the presence of this enzyme in bovine milk, where it is part of the microsomal particle. It is also found in the milk of sheep and goats, but is absent from the milk of sows and mares (12). Human milk is virtually devoid of the enzyme.

Enzyme absorption

The pharmaceutical probability that orally administered enzymes may be absorbed rather than digested has been shown in the case of chymotrypsin (8). Demonstrable increases in blood levels of chymotrypsin and similar acting enzymes were noted and their clinical efficacy has been utilized in many cases of trauma and blood extravasation.

MICROPARTICLES IN NUTRITION

In his studies on the absorption of microcrystals, Reimann (23) discovered microparticles in heart muscle and photographed them with polarized light. In the generic drug controversy, it was shown conclusively that certain pharmaceutical preparations, though of identical chemical structure, exhibited vastly different levels of biological availability. Most of these well-publicized diversities were caused by variation in particle size and by excipients added to the final pharmaceutical preparation. Micronization of drug particles in the generic preparation as well as in known pharmaceuticals resulted in a higher blood level of the active substance (30).

Principles of pharmaceutical preparation should be extended to foods and their additives. Micronization of the ingredients of a particular food would produce a higher blood level of those ingredients than would be possible with the original particle size. Most foods are chewed, and their particles are digested. There is, however, one almost universally consumed food that has been micronized, especially since 1938 in the U.S.A. That food is bovine milk.

Since bovine milk contains active xanthine oxidase even after pasteurization, homogenization (micronization) of the milk produces at least 3.5 times more particles 1 μ or less in size than are present in unhomogenized milk (4). Although the quantity of xanthine oxidase may be identical in pasteurized milk, pasteurized-homogenized milk, and curdled milk, the enzyme's biological availability would be highest with the microparticle-laden homogenized milk and almost absent from curdled milk with its preponderance of large size particles. On this basis, we would have to discount the belief generally accepted by

nutritionists that only the caloric value and chemical content (carbohydrates, proteins, and fats) of foods are important and then consider the preparation and particle size of foods as being important in their biological effects.

Shaper (28) compared blood cholesterol levels of two nomadic tribes in East Africa: the Samburu, who consume bovine milk and have a relatively low serum cholesterol value of approximately 190 mg%, and the Rendille, who drink camel's milk and present a serum cholesterol pattern that closely parallels that of "Western" groups, peaking to 259 mg% at age 55. Here again, particle size may be helpful in explaining the disparity. It is known that camel's milk contains considerable numbers of fat globules of such small size that, despite prolonged churning, it will not yield any great amount of butter.

EPIDEMIOLOGICAL STUDIES

Epidemiological studies concerned with the incidence of atherosclerosis that rely on death rates, number of myocardial infarctions, electrocardiographic findings, and serum cholesterol determinations are statistically unreliable. Undiagnosable atherosclerotic changes and myocardial scarring are present in many more people than the unfortunate number who exhibit often fatal manifest myocardial infarction. Autopsies of young males that have died from war wounds have proven that changes in the arterial wall may be present without causing any circulatory symptoms (5). Among the persons afflicted with atherosclerosis or myocardial scarring, only a limited number suffer actual heart attacks. Many reach a ripe old age despite the presence of extensive changes in the arterial wall or of myocardial involvement, and are not considered in statistical surveys. While bearing in mind these limitations,use was made of published statistics on death rates from myocardial infarction as well as data on the consumption of cows milk, in order to pursue the theory of ectopically deposited xanthine oxidase absorbed in microparticle size from ingested cow's milk. The countries selected for inclusion in the following table were those that occupy extreme positions (low or high) in the scale of reported death rate and extremes in bovine milk consumption. Unlike in other studies, the actual number of deaths is considered arbitrary and without relevance to the statistical accuracy. Therefore, only ranges are utilized.

The Japanese, as well as other oriental populations, do not consume bovine milk in any significant amount. Their rate of death from myocardial infarction is low. The Swiss reportedly drink mostly milk that has been boiled, a process that would inactivate xanthine oxidase; here too, the death rate from myocardial infarction is only moderate. Although milk consumption in the United States is almost identical with that of the Swiss, the death rate from myocardial infarction in the former is on the upper end of the scale, nearly doubled. The United States population consumes milk in homogenized form, and the biological availability of the xanthine oxidase contained in homogenized milk is

Table II

Death Rate from Myocardial Infarction Correlated with Milk Consumption of Various Populations

Country	Death from myocardial infarction	Daily milk consumption (ml)	Particle size milk preparation(μ)	Xanthine oxidase content Lück units*
Japan	Very low	55	1–20 Powdered + homogenized	9 (powdered) 7 (homogenized)
Switzerland	Medium	320	1–20 Raw, boiled milk	0 inactivated
United States	Very high	375	1–5 Homogenized milk	7 (factor 3.5)
Finland	Very high	850	1–20 Pasteurized (+ homogenized)	13
East Africa (Masai)	Almost nil	7000	Macroparticles curdled milk	None biologically available

* 1 Lück unit = $\frac{2500}{t}$, where t = time for decolorization of methylene blue; 2500 = a complex factor related to the number of μmole of xanthine oxidized /min/mg protein at 25°C.

at least 3.5 times greater than that of unprocessed milk. Therefore, the average daily consumption of homogenized milk in the United States is actually the equivalent of approximately 1,300 ml of unhomogenized milk, when the biological availability of milk xanthine oxidase is considered. The Finns report an extremely high milk intake per person, with only some of it homogenized. Their death rate from myocardial infarction is equal to and even surpasses that of the United States.

The Masai of East Africa are the bane of the protagonist of the hyperlipidemia-induced atherosclerosis theory. This well-financed and well-publicized concept is based on data gained from artificially created conditions in animals rather than on conditions found in this tribe of humans who would provide an exceptionally good source material for studies of high saturated fatty acid intake with no resulting atherosclerosis or hypercholesterolemia. These people ingest 7 l or more of curdled milk daily, almost exclusively; thus, saturated fats constitute about 60% of their diet. Curdled milk is characterized by large-sized particles and no biologically available xanthine oxidase. In similar fashion, many high-milk-consuming population groups prefer soured milk or boiled milk to raw or processed milk.

Just as Reimann (23) demonstrated the presence of microparticles in human heart muscle, we can postulate that microparticles of xanthine oxidase in

processed milk may be absorbed and deposited ectopically in the heart muscle. Small particles, absorbed from the intestines, are carried via the lymph stream into the subclavian vein, the right heart, the pulmonary circulation, the left heart, and then into the aorta and the generalized circulation. Substances absorbed in this way bypass the liver and enter the arterial system first. Xanthine oxidase finds a ready substrate, plasmal, from plasmalogen *in situ* in the heart muscle and in the arterial wall. Plasmalogen is present in the body in very small amounts during infancy, but increases with the aging process. Therefore, this chemical reaction will not take place in the early development stages when most milk is consumed.

Although normal human blood serum does not contain xanthine oxidase, preliminary findings by Ross *et al.* (26) revealed that the enzyme may be present in humans in the atherosclerotic plaque of the arterial wall and in heart muscle adjacent to the coronary arteries. It is known that human serum contains an inhibitor of the enzyme.

PAIN IN HEART DISEASE

The cause of the intense pain felt in angina pectoris and myocardial infarction has not been explained to anyone's satisfaction by the concept of ischemic heart disease (1). The plasmalogen-based explanation of heart disease may offer a plausible explanation for the origin of the pain. It would be ascribed to the algesic property of the enzyme phospholipase A (6, 10). The pain would be noticeable if the enzyme were to be deprived of one of its substrates, *e. g.*, diminution of plasmalogen in areas of the heart muscle by ectopic xanthine oxidase.

This theoretical consideration was tested by Oster (16), using the xanthine oxidase inhibitor allopurinol in numerous patients suffering from angina pectoris at rest. A quantitative dose response occurred, resulting in a reduction of the severity and frequency of the anginal attacks. The rationale for this treatment arose from the assumption that ectopic xanthine oxidase acts as a constant oxidizer of plasmal liberated from plasmalogen, causing it to disappear completely. Phospholipase A *in situ* in the heart muscle may be activated by calcium ions, catecholamines, or other, as yet not well-known, influences (10). The inhibition of anginal pain by propranolol, a blocker of beta adrenergic receptors, may then be explained by the blocking of the catecholamine influence. These mechanisms are shown schematically in figure 1.

Still unexplained is the mechanism of action of nitroglycerin in the alleviation of anginal pain. Neutralization of phospholipase A, or the inhibition of its effect on plasmalogen, or, lastly, its potential combination as nitrite ion (supplied by nitrate ions) with catecholamines, resulting in the formation of nitrosamines, are chemical possibilities that are under investigation.

INCONSISTENCIES IN THE HYPERLIPIDEMIA RATIONALE

There is a known difference between the incidence of myocardial infarction in women of child-bearing age and that in males of the same age group, the ratio being about 1 : 8. This disparity exists despite consumption of the same diet by males and females, and although both sexes are subject to similar mental stresses. Plasmalogen has been found to be a target substance for sex hormones (13). It is quite possible that the cyclical changes found in such tissues as the rat kidney may also occur in human heart muscle. In the female, therefore, with the changing hormone levels during the menstrual cycle, some of the plasmalogen depleted by the action of ectopic xanthine oxidase may be restored to the heart muscle cells. However, proof of this assumption is almost impossible to obtain. Atherosclerotic lesions found in both sexes are described as almost equal in all age groups (24).

The spotty distribution of so-called ischemic lesions is extremely difficult to explain by the concept of mechanical origin of these lesions. The ectopic xanthine oxidase theory would provide a plausible explanation also for the origin of ectopic foci causing arrhythmia. Forming part of the cell membrane, plasmalogens are in a strategic location to control cell permeability. One might postulate that a disappearance of plasmalogen from the cell membrane may cause a loss of equilibrium between endocellular potassium and ectocellular sodium, resulting in a change in the electrical potentials, which may be recorded electrocardiographically and act as new foci of heart rhythm control. The sarcolemmal membrane is also most probably the site at which many of the drugs that affect the function of the heart exert their influence (20). Proved *in vitro,* plasmal in the cell membrane may also act as receptor for many drugs (19).

APPLICATION AND CONCLUSIONS

There is a basic species difference in the xanthine oxidase content of human and bovine milk. The author has attempted to show that this enzyme, when absorbed in small particle size from the intestinal tract, may react with an essential and strategically situated component of the cell membrane, the plasmal moiety of plasmalogen. Lesions created by this chemical reaction may represent the first damage to the cell integrity that requires repair. Repair mechanisms can vary in different locations: scar formation in the myocardium, and possible cholesterol and fibrin infiltration in the arterial wall. This concept would create a new disease entity, the *Plasmalogen Disease,* resulting from localized phospholipid depletion, as opposed to the concept of tissue damage allegedly caused by a plethora of lipids in the circulating blood.

The augmented biologically available xanthine oxidase in homogenized bovine milk could conceivably be viewed a "food additive" capable of effecting

tissue changes that would occur simultaneously in the myocardium and in the arterial wall, both plasmalogen-containing tissues. These pathological events would have their onset in youth, when so much bovine milk is consumed long after weaning, leading to the creation of fatty streaks and culminating in full-fledged atherosclerosis and myocardial scarring in the later decades of life (7).

The proof of this new concept of the genesis of atherosclerosis as an expression of plasmalogen disease is difficult to achieve and does not derive from often misleading results of blood analysis. Significant results may be obtained from tissue examination, pharmacological results of enzyme inhibition studies, and epidemiological investigation of milk consumption and usages of various population groups.

The concept of the mechanical origin of heart disease is under serious challenge (3). Application of this theory to the treatment of myocardial disease has afforded only symptomatic improvement of the victims. Prevention of myocardial infarction by attacking the presence of hyperlipidemia has also encountered adverse criticism. The proponents of this unproven theory, which was promoted chiefly by the dicta of the American Heart Association, advise *ex cathedra* the absolute avoidance of whole milk, butter, and cheese, completely disregarding that the recommended diet is unrealistic and unacceptable to most people. This unfounded attack on the consumption of milk and milk products spawns such wasteful developments as 99% fat-free homogenized milk, which was found to have the same xanthine oxidase content in microparticle size as the normal pasteurized variety (25). The recommended eschewal of dairy fat consumption by population groups is abetted by commercial interests (9).

Application of the expounded theory of ectopic xanthine oxidase affecting the plasmalogen content of myocardial cell membranes would be quite simple to accomplish as a public health measure. Such a step might lower the United States level of myocardial infarction and atherosclerosis to that found, for instance, in Switzerland, representing a reduction of at least 50%. The technological pseudoadvancement of milk homogenization should be replaced by making available to the consumer a biologically active xanthine oxidase-free milk resembling human milk. This proposal would be realistic, even though the results cannot, as yet, be fully guaranteed by the data at hand. In these times of doubt, when the biological effects of food additives, pesticides, and air pollutants are being subjected to question, it would be relatively simple to eliminate a food additive that is produced as a result of various forms of processing.

The proof of the theory would be forthcoming if it could be shown that youth nourished with biologically active xanthine oxidase-free milk exhibit almost no fatty streaks in their arteries and, in later age, display fewer and lesser secondary effects of atherosclerosis and heart disease. At present, the author is

attempting to shortcut this rather lengthy process by giving patients with hyperuricemia and angina pectoris at rest another known xanthine oxidase inhibitor, folic acid. Preliminary results have been favorable; elevated uric acid levels were reduced on a dose-response basis, and the frequency of anginal attacks was diminished, although not as spectacularly as with the use of allopurinol. When two different chemicals having one target effect in common, namely inhibition of xanthine oxidase, are shown to also produce the same clinical effect, this should be considered a "giant step" forward in the pharmacological proof of a new concept of the genesis of some heart diseases.

SUMMARY

A biochemically plausible mechanism of the cause of some heart diseases is presented, based on plasmalogen metabolism. The importance of particle size to intestinal absorption is applied to explain the possible dietary origin of both atherosclerosis and heart disease. Such observations as the lower incidence of heart attacks in women of child-bearing age, absence of atherosclerosis in the Masai, cause of anginal pain, absence of coronary artery narrowing in a high percentage of angina pectoris patients, all of which are unexplainable by the hyperlipidemia theories, may well be clarified by the plasmalogen-phospholipase-xanthine oxidase interaction concept of cardiovascular disease. An urgent appeal is made: 1) to immediately discontinue homogenization of milk; and 2) to create a biologically active xanthine oxidase-free milk for the consumer.

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Original Article

Treatment of Angina Pectoris According to a New Theory of Its Origin

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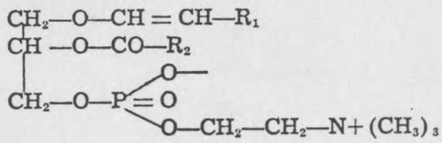
In a recent case of fatal myocardial infarction a selective disappearance of plasmalogen from heart muscle cells was noted.[1] This observation, together with prior studies on the distribution and the physiologic behavior of plasmalogens, has led to a new theory of a possible cause of myocardial infarction and angina pectoris.

Plasmalogen, discovered in 1924[2] was first considered to be an acetal phosphatide.[3] Later it was found that an unsaturated fatty acid is also a constituent of plasmalogen, and the following formulation is now generally accepted:



R_1=fatty aldehyde residue
R_2=unsaturated fatty acid residue

Investigators[4] demonstrated that choline plasmalogen is prevalent in heart muscle but is found in only small amounts in the brain and may act as a substrate for phospholipase A (lecithinase A) which is known to hydrolyze lecithin.[5]

Plasmalogen and a phospholipase A-like enzyme are usually present in normal heart muscle. The enzyme

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2. Feulgen, R., & Voit, K., *Pfluegers Arch. Ges. Physiol.*, 206:389-410,1924.
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may be activated by calcium or by catecholamines, effecting a hydrolysis of plasmalogen, resulting in the formation, as split products, of plasmal plus a combined form of glycerophosphorocholine with an unsaturated fatty acid. In normal hearts this process would be continuous and reversible. Considering the cited disappearance of plasmalogen from heart muscle, it may be concluded that if hydrolysis becomes unbalanced by an excess of enzyme activity, plasmalogen would then disappear from the affected area and subsequent cell death, or infarction, would result. In angina pectoris the hydrolytic process is quantitatively lesser, resulting in a probable diminution rather than in a disappearance of plasmalogens. Free phospholipase A, deprived of its substrate, is a powerful algesic and could be a cause of the severe pain experienced in angina pectoris and myocardial infarction.[6]

Experimental proof of the foregoing theory is inherently difficult, since the site of the chemopathologic processes is the heart muscle cell. These processes are herewith offered as a possible explanation for those cases of myocardial infarction (approximately one third of the known total) for which none of the commonly accepted reasons for infarction, such as severe coronary atherosclerosis or coronary thrombosis, can be found despite thorough tissue investigation. Admittedly, animal experiments do not always simulate human pathology and may, furthermore, lead to therapeutic attempts at correction of the yardstick of the disease rather than the disease itself, a substitution now encountered in the current efforts to depress serum cholesterol levels.[7] To prove the author's plasmalogen theory with special emphasis on its postulated reversible form in angina pectoris, attempts were made to restore the depleted plasmalogens to the heart muscle. If such a plasmalogen restoration could be accomplished by the use of drugs with no known vasodilating, reoxygenating, or lactic acid-neutralizing action, thus ruling out prevailing theories of drug therapy, it should be considered meaningful in support of the plasmalogen-based theory.

Prolonged observation of plasmalogen distribution in animals and humans has made the author acutely aware of three distinct distribution qualities of these compounds: (1) their selective appearance in only some of the same functional units within an organ, e.g., kidney; (2) their response in distribution in certain anatomic positions to the influence of male and female hormones; and (3) their almost complete absence from normal liver cells.[8] This last observation was made the fulcrum of the new approach to angina pectoris therapy. It had been demonstrated that xanthine oxidase, normally present in the liver, was capable of oxidizing plasmal and thereby contributing to the oxidation of plasmalogens and their absence of histochemical demonstrability in normal liver tissue.[9] It was presumed that prevent-

6. Meldrum, B. S., *Pharmacol. Rev.*, 17:393-445, 1965.

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9. Oster, K. A., & Mulinos, M. G., *J. Pharmacol. Exp. Ther.*, 80:132-138,1944.

ing xanthine oxidase from oxidizing plasmalogen would increase the concentration of these substances in the blood and would effect their restoration to the depleted areas in the heart muscle in cases of angina pectoris. Creation of such a liver-heart axis should then lead to a diminution of angina pectoris attacks, especially those unrelated to effort, emotion, or food intake, the so-called decubital or nocturnal angina.

Since the theory demands that an appreciable quantity of plasmalogen leave the liver in its unoxidized state, the influence of any drug designed to bring about this effect must depend, of necessity, on dosage-response. There must be sufficient blockage of enzyme action in the liver to produce enough plasmalogen for effective restoration to the depleted areas of the heart.

The ability to inhibit xanthine oxidase demonstrated by the drug allopurinol* has led to its recent introduction to the medical armamentarium specifically for the prevention and treatment of gout. Although it has been claimed that allopurinol does not inhibit the oxidation of smaller aldehyde molecules, it was deemed worthwhile to titrate the action of this drug, which has no known effect on the vascular system and the heart, on patients suffering from angina pectoris.

The response of angina pectoris to drugs is notoriously unpredictable. However, to date, four patients, three women and one man, have been treated with allopurinol with amazing success. The need of these patients for nitroglycerine was definitely diminished. The drug was given in increasing amounts to rule out placebo effects. With a 200 mg. dosage (two, 100 mg. tablets daily) there was no change reported in either the frequency or severity of the attacks. Essentially the same lack of response was obtained when the dose was increased to 400 mg. daily (four, 100 mg. tablets). However, after the introduction of a daily 600 mg. dosage, two tablets of 100 mg. three times a day, all patients reported an unquestionable diminution of the angina pectoris attacks, both as to severity and frequency.

*Zyloprim®, Burroughs Wellcome, Tuckahoe, New York.

Case Reports

Case 1

A woman aged 58, had experienced for about one year daily attacks of angina pectoris at approximately 5:30 a.m., which were relieved three to four minutes after sublingual application of nitroglycerine. Since she usually experienced some additional chest pain during the day, her average nitroglycerine intake was eight to ten tablets daily. The angina attacks were not curtailed by short-acting coronary vasodilators nor by the long-acting modifications. For many years this patient had a hypercholesterolemia ranging from 330 to 430 mg. per cent. Her weight and blood pressure were normal.

On January 30, 1967, the patient was instructed to take one-100 mg. allopurinol tablet twice daily. On February 24, after 3 and one-half weeks on this dosage she reported no relief from her angina pectoris attacks. At that time the dosage was increased to 100 mg. allopurinol four times a day. Two weeks later, on March 10, she stated that there was no appreciable change in her chest pain. She had experienced, however, some nocturia as a new symptom. Allopurinol was now increased to two tablets of 100 mg. three times daily, a total dose of 600 mg. each day. Until this time the patient had been taking erithryltetranitrate four times a day. This medication was now discontinued. On March 20 after 10 days on the 600 mg. dosage, she claimed that for the first time her attacks were of greatly diminished severity, though still as frequent as previously. By March 24 the attacks were definitely of lessened severity.

Her serum cholesterol at this time was 340 mg. per cent.

On April 7, still on 600 mg. allopurinol daily, she stated that she required but two to three nitroglycerine tablets a day, in contrast to her previous daily consumption of eight to 10 tablets. The patient was still awakened by chest pain in the early morning, but she described the pain as being so short-lived that it subsided almost before she could reach the nitroglycerine tablets on her night table, happily comparing this situation with the previous three to four minute interval of pain between administration of nitroglycerine and the onset of relief. She also reported the ability to continue normal activity even when taking nitroglycerine during the day. In the past she had found it necessary to sit down and wait until the attack of pain had subsided.

This patient continued on allopurinol 600 mg. daily with the same relief of symptoms. When a reduction of the dose to 400 mg. a day was tried, the angina pectoris again became so severe that the patient herself, on the fourth day of the reduced medication, resumed the 600 mg. daily regimen, with relief of her symptoms. Incidentally, her serum cholesterol rose to over 400 mg. per cent during this period with no evident aggravation of her symptoms. A double-blind study of the administration of the drug produced an alarming recurrence of severe angina pectoris pain lasting for about 20 minutes and responding poorly to nitroglycerine. This occurred during a period of placebo medication.

Case 2

A woman of 68 had suffered for many years with auricular fibrillation and had experienced several attacks of pulmonary edema. Therapy included quinidine, digitalis, and diuretics. In April, 1967, she was hospitalized with acute pulmonary edema which was successfully treated. However, the patient experienced severe attacks of morning angina pectoris for which sublingual nitroglycerine was required. When she was given allopurinol 100 mg. four times a day there was no change in her condition. However, with administration of two, 100 mg. tablets three times daily (600 mg. total) the angina pectoris did not recur. Following release from the hospital this patient was instructed to reduce her daily allopurinol intake to 400 mg. with resultant prompt recurrence of morning angina pectoris. Once more, increasing the daily dosage to 600 mg. caused relief of her painful attacks. Her usual serum cholesterol concentration of approximately 300 mg. per cent remained unchanged by the administration of allopurinol.

Case 3

A man aged 65 experienced angina pectoris with mild exertion and sometimes even when at rest. His weight was normal, blood pressure 140/80, and pulse rate 76 with regular rhythm. As of October, 1966, his nitroglycerine requirement was 40 to 60 tablets per week. His serum cholesterol level was 170 mg. per cent and the ECG showed a 1° AV block. Placing him on isosorbide dinitrate therapy resulted in a reduction of his nitroglycerine intake to about 10 to 12 tablets per week. Nevertheless, the patient still complained of substernal "burning," which was ascribed to a hiatus hernia. On April 24, 1967, treatment with allopurinol was initiated, 400 mg. daily in four divided doses. By May 1, his nitroglycerine intake had been reduced to four tablets for that week. The patient claimed great improvement, and, when seen on May 15, he had gained six pounds in weight as a result of improved appetite following the complete disappearance of the substernal burning sensation.

Case 4

A woman of 62, suffering with hypothyroidism, had experienced attacks of angina pectoris since before she was first seen by this investigator in April, 1958. She had been treated with every conceivable drug and drug combination for the relief of her thyroid deficiency, hypercholesterolemia, and cardiac weakness. Most recently, she has been taking digoxin 0.25 mg. and desiccated thyroid 0.065 Gm. Long-acting nitrates had brought very little relief from her anginal attacks. This patient, of normal weight, had the following blood values before commencing allopurinol therapy: serum cholesterol 370 mg. per cent, uric acid 5.6 mg. per cent. Her daily nitroglycerine requirements averaged six tablets daily. On May 29, 1967, she was placed on 600 mg. allopurinol daily. When seen a week later, on June 5, the patient claimed that she felt definitely better and needed only an average of three nitroglycerine tablets daily. On the third day she felt no chest pain whatever and, consequently, did so much work, of the type she was previously unable to perform, that she required five nitroglycerine tablets the following day.

Five additional patients were studied, with similar results, since this paper was prepared.

Discussion

Although the relief of anginal

pain by allopurinol does not prove the plasmalogen theory per se, it was the pursuit of this mechanism which led to the use of the drug. The inferential assumption that allopurinol, by inhibiting xanthine oxidase, increases the blood plasmalogen level is now under investigation. Base values for plasmalogen in the blood will be determined in a larger population group, with all the possible variations owing to drugs, food intake, and age. Allopurinol was designed to inhibit uric acid metabolism. It should be understood that this drug was chosen for use in this investigation only because it was an available nontoxic xanthine oxidase inhibitor. It must surely be realized that there may well be other chemicals with capabilities more specific than that of allopurinol for inhibiting the aldehyde oxidizing qualities of xanthine oxidase. There may also be therapeutic approaches to the restoration of plasmalogen other than inhibition of xanthine oxidase once the plasmalogen theory of angina pectoris and myocardial infarction has found more proof. Angina pectoris and forms of myocardial infarction might be manifestations of plasmalogen diseases, which may also be encountered in other histologic locations of the human body.

The reported difference in plasmalogen distribution between male and female animals and its relationship to gonadal hormones may furnish an explanation of the statistically proven fact that women of childbearing age have such a lower incidence of manifest myocardial infarction than men in the same age group. Plasmalogens and cholesterol are known components of the cell membrane.[10]

The complex mechanism of pain production in angina pectoris has been ascribed to vascular spasm of the coronary arteries and to production of lactic acid due to poor oxygenation of the myocardial cell. However, neither of these theories explains the pain-relieving action of nitroglycerine in angina pectoris. The theory of plasmalogen depletion in the heart muscle as a possible cause of myocardial pain requires the unopposed action of phospholipase A on plasmalogen according to the equation:

Activated by
Calcium Catecholamines
↓
Phospholipase A
↓
Plasmalogen
$(+H_2O) \downarrow\uparrow (-H_2O)$
Plasmal + Lysolecithin

Since phospholipase A is an algesic compound, its action without sufficient substrate may be the cause of some of the angina pectoris pain. Restoration of the balance of plasmalogen would then furnish enough substrate for phospholipase A to act on and thus prevent its algesic effect. It is not known if phospholipase A is present in the heart muscle in the form of a proenzyme. It is also not known if nitroglycerine inactivates or neutralizes the activity of phospholipase A. However, it is known that this enzyme may be ac tivated by catechol amines and by calcium ions.

Proceeding with the concept of the outlined theory, the destruction

10. Cuthbert, A. W., *Pharmacol. Rev.*, 19:59-106,1967.

of plasmalogen by xanthine oxidase, occurring normally in the liver, is prevented by the introduction of a xanthine oxidase inhibitor. Allopurinol, a xanthine oxidase inhibitor, was given to patients with known heart disease, and in some patients also hypercholesterolemia, who were sufferers from angina pectoris. There was a definite dosage and response relationship before the angina attacks subsided in frequency and severity. In the language of the proposed theory enough plasmalogen had to be restored to the heart muscle before the action of phospholipase A could be opposed and neutralized. Instead of a diminution of phospholipids in the blood stream which is the aim of most therapeutic endeavors at the present time, an increase of selective phospholipids was here attempted to relieve anginal pain. This was brought about by action on the liver. This organ, when damaged by alcoholic liver cirrhosis, is known to reduce the instance of myocardial infarction by about 80 per cent.[11] Some drugs currently in use for cardiovascular illness act primarily on the liver for their effectiveness, as, for example, the anticoagulants, which alter the prothrombin formed in the liver. Thus, there may well be a relationship of heart to liver in the cause and possible prevention of heart disease. To speculate on the latter, one could visualize a xanthine oxidase activator contained in certain foods as responsible for an excessive destruction of plasmalogen and the cause of eventual myocardial disease.

The four patients studied had no hypertension or obesity, nor have they exhibited hyperuricemia or other manifestations of gout. There are reports that allopurinol-induced reduction of elevated uric acid in the blood of patients with hypercholesteremia and elevated serum triglycerides also caused a reduction of the triglycerides.[12] However, there has been no description of a connection between the action of allopurinol in patients with normal uric acid metabolism and action on the heart, nor have any significant findings of interrelationship of high serum uric acid and angina pectoris been reported.

Summary

A new theory has been postulated as to the cause of some myocardial infarction and angina pectoris. The theory involves an interplay of plasmalogen plus its hydrolysis by phospholipase A to plasmal and lysolecithin. A new therapeutic approach for the treatment of angina pectoris by using xanthine oxidase to inhibit the normal metabolism of plasmalogen in the liver is described. Allopurinol, a xanthine oxidase inhibitor, was successfully used in four cases of angina pectoris to reduce the frequency, duration, and severity of the attacks. A double-blind test was utilized to verify the results obtained. ■

11. Hirst, A. E., et al., *Amer. J. Med. Sci.*, 249:143-149,1965.

12. Berkowitz, D., *J.A.M.A.*, 190:856-858,1964.

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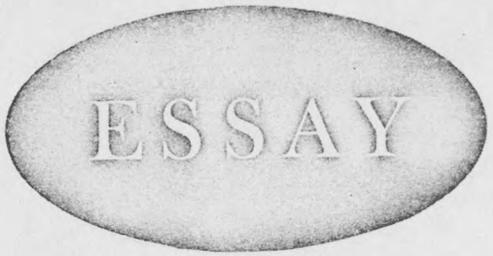

Bovine Milk Xanthine Oxidase as one of the Dietary Causes of Early Atherosclerosis

Kurt A. Oster, M.D.

My article in *Medical Counterpoint* (November, 1973), "Is an Enzyme in Homogenized Milk the Culprit in Dietary-Induced Atherosclerosis?" has elicited much response. Some of the letters which have been printed by this journal (May, 1974) contain queries which I shall attempt to answer. In particular, I feel impelled to respond to some of the unfounded and misleading statements by M. F. Brink, Ph.D. of the National Dairy Council.

The article should have included an important footnote, namely, that it was a resumé of an address I had made in Nairobi, Kenya, September, 1971, at the invitation of the East African Medical Research Council, which will be published in a volume of proceedings of that symposium on preventive myocardiology and cardiac metabolism. This might have countered the rather unfair remark by Dr. Brink that I had misinterpreted the research of those who had "actually conducted the studies." I merely quoted the far-reaching generalizations of one group of investigators who, after studying 23 young Masai warriors, ascribed to them special genetic qualities. The opposite results with another group of 24 young Masai were published by Mann *et al* in May, 1974. I leave it to the wisdom of Dr. Brink and his group of experts to reconcile these differing results with the same people.

I posed the questions: why do the Masai, those thousands of humans who consume 60% of their diet as saturated fat plus plenty of cholesterol, not exemplify the various equations and dire predictions of the researchers sanctioned by the American Heart Association? Any why do they not have such a degree of atherosclerosis and subsequent myocardial infarction because of that diet to head the list of "risk-factor"-threatened humans? This doubtful honor belongs to the East Finns. Dr. Brink simply excuses the Masai as being "not free of emotional stress." What particular insuperable stress do the agricultural Finns of Eastern Karelia have to merit such a high heart attack rate? Some tell us that the Masai, nomadic herders, walk such great distances that high caloric expenditure protects their hearts. Again in contrast, the woodsmen and lumberjacks of East Finland, who expend enormous energy in their jobs, are not so protected. These observations simply do not fit the laws of risk factor epidemiology so frequently quoted to lull the non-curious mind into believing that they may be the scientific truth, which they are not.

I went to Kenya not to analyze the arterial trees on the serum cholesterol of the individual Masai. I simply wanted to know if the milk consumed by this group of people is similar to ours. With much inquiry and sifting of misinformation, I finally found that the Masai drink only a kind of soured milk similar to milks consumed by many other pastoral people with no facilities for prolonged storage. According to my informants, the Masai store their milk in gourds cleaned with sterile cow's urine.

At about that time, I arrived at the conclusion that the dietary changes encompassing low saturated fat intake foisted upon the American people by the American Heart Association are based on false premises, poor observation, and insufficient experimentation and are governed by misleading propaganda; they simply do not

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Photograph courtesy of David Stecher, Westfair Center, Westport, Conn.

jibe with observed facts.

There was never any evidence that souring of milk destroys its xanthine oxidase, as Dr. Brink states. Nor have I ever made that contention. What I did state is that the absorption of such a macromolecule as xanthine oxidase is not only possible but also enhanced by *homogenization* of the milk fat droplets which carry the xanthine oxidase. Soured milk particles are large and subject to the digestive influences of our intestinal proteases. The xanthine oxidase carried by these larger particles is thus more apt to be destroyed and digested than the xanthine oxidase molecules carried by the extremely small fat micelles present in our homogenized milk. Further, the different intestinal flora of these fermented milk drinkers might in some way protect them against faulty absorption.

Dr. Brink argues that experts in the "fields of enzymology, biochemistry, and medicine do not believe that such a large protein molecule can enter the digestive tract and pass through it intact into the blood without being broken down by the digestive enzymes." These anonymous sources, informants of the National Dairy Council, must be oblivious to the voluminous literature on macromolecular absorption beginning as early as 1924 (a Harvey Lecture by Schloss) and continuing until as recently as May, 1974, in a paper by Warshaw, Walker, and Isselbacher. If they are in need of pertinent references, I refer Dr. Brink and his learned staff to my publication in *American Laboratory,* August, 1974, as well as to the observations of Davies *et al* in *Lancet,* May 1974. They will learn that the possibility of large protein molecule (macromolecule) absorption definitely exists. We have found in human sera specific antibodies to bovine xanthine oxidase. High antibody titers were exhibited by those patients with demonstrable manifestations of atherosclerosis. Such antibodies, as even the National Dairy Council must surely know, develop *only* in response to the *presence* of antigenic substances, in this case an absorbed foreign protein, xanthine oxidase.

I cannot fathom why Dr. Brink, as scientific spokesman for the milk industry, does not join me in my defense of milk as a wholesome food when free of a potentially damaging enzyme, xanthine oxidase. He evidently prefers to be aligned with those who condemn the saturated fat content of this nutritious food and who at this time have the majority of the research funds, the assistance of money gathering organizations and the glitter of pseudoscientific nutritional studies investigating the cardiac death rate and the vagaries of serum cholesterol levels of small population groups with ambiguous and inconsistent results.

Both Dr. R. T. Whalen and Dr. Brink comment on my therapeutic effects with pharmacological doses of folic acid. I am presently giving folic acid to approximately 80 patients with such gratifying results in diminishing the progress of atherosclerosis—and with a complete absence of toxic effects—that I applied to this compound the epithet of "penicillin equivalent for atherosclerosis" in a 1973 address before the American College of Clinical Pharmacology.

Again, I regret the blatant literature ignorance expressed by Dr. Brink when he said he has "not seen evidence evaluating the purported therapeutic effects of

folic acid, particularly with regard to the claimed effects of this substance in peripheral vascular disease." I suggest that he search the American patent listings to find a patent issued in 1969 to Dr. Tibor Kopjak for the special therapeutic effect of folic acid, particularly with regard to its effects in peripheral vascular disease. I am most willing to provide him with additional information, should he and his aides remain blind to the published evidence of this compound as a xanthine oxidase inhibitor. He might then realize that the soundness of my publications in the scientific literature is based on fact and that I am proud to have been the first to identify the relationship of xanthine oxidase and plasmalogen depletion. Progress in research can be accomplished only with an open mind and a critical attitude to unsubstantiated propaganda.

Dr. S. Hershberg's suggestion that the destruction of bovine xanthine oxidase could be accomplished by the pasteurization process must be answered in the negative. Full destruction is accomplished by higher temperatures (81–84°C for 3 to 5 seconds) than those routinely used for pasteurization. Since the dairies do not generally produce such a xanthine oxidase-free milk, I suggest to the mothers of this country that they bring all commercially available milk to a simmer and then recool it before use. I believe that this simple process will prevent absorption of most of the xanthine oxidase contained in milk and thus aid in the prevention of the early atherosclerosis which threatens our American youth. Care should also be taken to avoid those ice creams which contain xanthine oxidase in large amounts. Absorbability of the enzyme is enhanced by the presence of emulsifiers commonly added to this food so ubiquitously consumed by our youth.

The dairy industry should provide the consumer with a milk which maintains its nutritional values of fats, proteins, and sugars and strive to eliminate not only pathological organisms but also all species specific enzymes which might be potentially harmful to humans. One should remember that one liter of whole milk contains about 120–160 mg of xanthine oxidase. If, in its daily consumption, only 1 or 2% of this foreign protein were absorbed, the cumulative biological effect could be enormous and frightening as a health hazard.

My concern at this time is with exposing a chemically plausible dietary cause of the initiation of the atherosclerotic process. I fully realize that there may be other endogenous causes. The results of my research have been reported in responsible medical and pharmacological publications for many, many years. The National Dairy Council should honestly investigate my claims, not condemn them *a priori* simply because they do not fit the scenario set by occasional self-serving interests concerned with losing face. Rather than attempting to refute my milk xanthine oxidase studies, it seems to me that the milk producers' ire should be directed toward those untenable claims of the American Heart Association that all Americans, not just the afflicted, should lower their serum cholesterol levels to enter the Nirvana of freedom from or reduction of heart attacks. Its magic wand would be substitution of artificial margarine for wholesome butter, consumption of skim milk instead of whole milk, avoidance of most cheeses, the restriction of eggs to three a week, etc. The unnecessary waste of costly food energy resulting from these unproven recommendations should be a concern of the National Dairy Council, just as it is to the American food consumer.

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In Answer to Your Letters. . .

Medical Counterpoint, August/September, 1974, carried three letters pertaining to previous articles of mine. The letters were most gratifying and posed a number of excellent questions, some of which I shall attempt to answer.

Dr. F. X. Schloeder asked about milk consumption in East Finland and of the physical characteristics of the milk. In 1968, the Finnish embassy informed me that the 1967 consumption of whole milk equivalent was 309.5 kilograms per person. 1967 sales of milk were 135 million kilograms homogenized and 388 million kilograms partially homogenized. Homogenization was start-

ed in Finland around 1956. Absorbable xanthine oxidase in butter and cheese? Butter contains no xanthine oxidase. Cheese proteins, being in large particle form, not emulsified or homogenized, the xanthine oxidase would more likely be digested than absorbed. Universal milk homogenization was begun in the United States about 1938.

By testing a specific antibody to bovine xanthine oxidase, we have determined that there appears to be great individual variation in absorption of the enzyme. In response to Dr. Schloeder's last question, there does seem to be a lower incidence of atherosclerotic disease in rural populations than in urban ones.

Dr. E. Henry Lamkin's letter posed several questions. My answers follow: bringing milk to a boil completely inactivates the contained xanthine oxidase. The enzyme is a protein and is contained in the protein layer surrounding the fat droplets of bovine milk. Homogenization breaks up the coarse fat droplets and creates different micellar structures. Since xanthine oxidase is also contained in microsomal structures, ordinary refrigeration will liberate the enzyme and may make it more biologically available, a phenomenon described by French investigators.

The properties of cream in relation to xanthine oxidase are variable. We have studied 67 samples of shelf milk products but have refrained from publishing the results because of the variability of commercial processing encountered. Therefore, I cannot answer with certainty the questions about cottage cheese, fondue, and powdered milk. It should be noted, however, that the enzyme is irreversibly destroyed on relatively short heating at about 90°C.

I have no knowledge if plasmalogen will restore itself in the absence of, or the inactivation of, xanthine oxidase. However, this is hoped for. Folic acid in pharmacological doses maintains the constant inactivation of the ectopic xanthine oxidase. We are presently investigating the possibility that folic acid contributes to the synthesis of phospholipids. I do hope that such openminded physicians as Dr. Lamkin will consider clinical studies in this field which shows so much promise.

As for Dr. James L. Turner's question regarding low cholesterol diets in children resulting in low I.Q's, one must consider that a low cholesterol diet, especially when overdone, usually results in reduced protein intake, which may well lead to malnutrition and poorer learning ability. Even the so-called "prudent diet" recommends seven eggs a week for children as opposed to four a week for adults. I did not find any of this information in the *American Heart Association Cook Book,* nor did I find any dietary directions for the elderly therein. The American public is swamped with do-it-yourself books which deal with various bodily functions but which are sadly lacking in cautionary advice as to limitations and dangers of utilization by the wrong groups and, in the dietary field, overcompliance by fanatics.

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clinical pearls

NATHANIEL SHAFER, M.D.

AT LEAST one-half of the patients with hyperparathyroidism have normal serum phosphate levels.

●

A RARE VARIETY of endocrine adenomatosis includes hyperparathyroidism, pheochromoytoma, and medullary carcinoma of the thyroid (Sipple's triad).

●

ALBRIGHT'S DICTUM should be noted: "Given a patient with hyperparathyroidism and a lump in the neck, the lump is probably not parathyroid." It is most likely due to a thyroid tumor.

●

THE MOST COMMON form of hypercalcemia seen today is that occurring in association with malignancy.

●

WITH ACUTE staphylococcal bacteremia, the chance of infecting at least one of the four heart valves may be as high as 60%.

●

IN TRICUSPID ENDOCARDITIS, particularly when superimposed on a normal valve, a murmur is likely to be absent.

RHINORRHEA ensues in 5% to 10% of all cases of head injury. It is usually transient, and the defect will usually heal spontaneously within a week in about half the cases and by a month in the vast majority of cases.

●

IN CONTRAST to dermatomyositis, the co-existence of scleroderma and malignant disease appears to be fortuitous, but the association of scleroderma with alveolar cell carcinoma of the lung is well recognized.

●

FEW PATIENTS with lupus erythematosis have the syndrome of chronic active liver disease.

Country	1967 Death Rate	Pounds Per Person Fluid Milk Intake	Pounds Per Person Butter	Pounds Per Person Cheese	Relative Standing M	Relative Standing B	Relative Standing C	Homog-enized	Pre Boiled Frequently
1. Finland	244.7	593	35.	7.3	1	1	12	⅓	No
2. United States	211.8	273	4.7	10.6	9	11	7	almost all	No
* 3. Australia	204.6	304	22.9	7.8	7	2	11	not generally	No
4. Canada	187.4	288	16.2	9.0	8	8	9	partially	No
5. United Kingdom	140.9	350	19.7	11.0	4	4	6	about 7½%	No
6. Netherlands	106.9	337	5.7	19.5	5	10	4	infrequently	?
7. F.R. of Germany	102.3	213	18.7	9.3	11	5	8	partially	?
8. Austria	88.6	327	13.2	8.4	6	9	10	occasionally	?
9. Sweden	74.7	374	16.3	18.3	2	7	5	?	Yes
10. Italy	78.9	137	4.0	19.9	6	12	3	12.5%	Yes
11. Switzerland	75.9	370	16.4	22.1	3	6	2	small quantity	Yes
12. France	41.7	230	19.9	28.8	10	3	1	negligible	Yes
13. Japan	39.1	48	?	?				occasionally	?

* School luncheon Milk all homogenized.

Guest Editorial

The Pragmatic Attitude in Preventive Medicine

Kurt A. Oster, M.D.

Recently I received a reprint of a chapter from "Progress in Cardiology," edited by Yu and Goodwin, 1973. The chapter was titled "Progress in the Epidemiology and Prevention of Coronary Heart Disease," by H. Blackburn, M.D. of the University of Minnesota School of Public Health. It contains some statements of interest to all physicians desirous of practicing medicine with intelligence, knowledge, and a critical attitude toward unproven, propagandized health proposals.

The author is very irritated by opposing ideas offered by others who, in his words, "are performing a disservice to the public, in sowing widely their misrepresentations." Another author's "polemic against unsaturates appears to be misleading and unnecessary. There are better ways of influencing the inaccurate health implications of margarine advertisements" which, incidentally, have followed the recommendations on diet given by the author's Minnesota group. Short shrift is given a third author: "I have had occasion to use this popular book as a classical illustration of self-deception in medicine." There are some pertinent observations about the spate of unsolicited health advice offered mostly in fear-inspiring books by authorities with impressive titles and academic credentials.

Dr. Oster is Adjunct Research Professor, Department of Biology, Fairfield University, Fairfield, Connecticut.

However, when Dr. Blackburn offers his own recommendations I shudder at the exposed dissonance, at the misuse and abuse of good words, and at the abysmal neglect of logical thinking. He counterpoints the academic attitude against the pragmatic attitude, pontificating for the latter rather than the conservative approach of testing suggested changes on groups of individuals, of finding out if the desired goal (reduction of serum cholesterol) will accomplish a reduction in ischemic heart disease, and, finally, if the proposed dietary changes might not present inherent risks (gallstones, malignancies, changes in brain development, etc.).

Dr. Blackburn follows the dictates of the Inter-Society Commission for Heart Disease Resources, which insists that something should be done. There should be no more "temporizing," and we all should eat the "prudent" diet (about 30% fat, in a ratio of ⅓ saturated, ⅓ monounsaturated, and ⅓ polyunsaturated fatty acids). He claims, falsely, (contradicting his own data) that this diet has been widely "tested" by millions who habitually use it in other cultures. He also claims, as does the Inter-Society Report, that "with these dietary principles, requirements for optimal nutrition can be met for all of the population, including infants, children, adolescents, pregnant and lactating women, and older persons."

That is quite a statement for an unproven remedy. It reminds me of the old-time snake oil salesmen who extolled their products' benefits for young and old, infants, pregnant women, etc. These travelling hucksters always promised that the excellence of their nostrums would be proven in the future. Dr.

Blackburn's group, with its multiple risk factor trials under way, has provided for itself the same escape route. They claim that "the programs *should* (emphasis mine) produce the definite evidence about prevention." We might well ask what will happen if the trials produce evidence contrary to the wishful thinking of Dr. Blackburn and his group. This has already happened in some coronary drug projects to the great chagrin of those who died as a result of using the prescribed cholesterol reducing agents.

Webster's International Dictionary, 3rd Ed., defines "pragmatic" as follows:

PRAGMATIC *(adj) 1: of or relating to the affairs of a community or state 2: active in affairs: busy;* officious, meddling *3:* stiff in one's opinion: conceited, opinionated, dogmatic *(emphasis mine) 4: practical, matter-of-fact 5: dealing with events in such a manner as to show their interconnection 6: a Kantianism: prescribing the means necessary to the attainment of happiness b: of or relating to the philosophic pragmatism of Peirce, James, and Dewey.*

Perhaps bits of definitions 2 and 3 have found their way into Dr. Blackburn's treatise.

Pragmatic concepts are often the antitheses of medicine's moral attitude. The pragmatist sadly lacks ethos and morality. His motto is "If it works it is good," provided it does not strike home. He might attain ephemeral acclaim if something is truly successful even without redeeming social value, but then we deal mostly with politicians and not physicians. A true physician tries to observe the eternal laws of nature, and with the understanding of these laws he provides better assistance to humanity.

The pragmatist may occasionally help an individual patient, but he has no place in preventive medicine for the masses, especially if he has never tested his concept on sufficient numbers. He will provide us with dogmatism, fanaticism, and conviction with missionary fervor but without rationalism. He will create a new set of rules based on faith. His followers may be endangered by overcompliance and differences in exegesis. The pragmatist will fight the critical attitude and stultify intellectual development. The danger of pragmatic pseudosolutions of "ut aliquid fiat" is real, particularly in these days of health maintenance organizations. The pragmatist will maintain the "health" or "non health" of the people by any placebo, by any expedient unproven method.

The pragmatist in professional standards review organizations is a danger. Dogmatic and stiff in his opinion, he will stymy inquiry and the creative mind, following the easy way of the majority, of the money resources. He is swayed by so-called authority or by the numbers of brainwashed believers. The pragmatist has already led us to targeted research. He will sound the death knell to universal knowledge and critical thinking. Creativity will be suppressed in favor of the attainment of pseudoresults which make good public relations and which facilitate the procurement of grant money and larger scientific emporia with more pushbutton equipment. When the crisis of the "Logos" arises, the catastrophic outcome will be a victory of the speculator over the thinker, the rule of the mob over the intellect.

Let us as physicians be coherent in our thinking, carefully weighing causes and effects and shunning unsubstantiated health schemes promoted under the flag of pragmatism. □

Prevention of Atherosclerosis: Fact or Fiction?

*A Critique of the Report of the Inter-Society Commission for Heart Disease Resources**

Kurt A. Oster, M.D.

EDITOR'S NOTE: *Dr. Oster's paper takes issue with certain conclusions of the Inter-Society Commission Report that have been drawn from the Framingham Study concerning the risk of coronary heart disease. It particularly challenges the stated relative effect on risk of CHD at different levels of serum cholesterol. Some of the questioning is based on clinical experience confirmed by others (e.g., the unimportant effect on serum cholesterol levels of significant reductions in dietary cholesterol).*

Moreover, Dr. Oster casts doubt on the value of epidemiologic studies of this type and questions the mathematical underpinnings of the statistical methods employed in this case. To review such matters we have called upon a consulting mathematician to offer a critique of Dr. Oster's remarks as well as the methodology of the Framingham Study. His comments immediately follow Dr. Oster's paper.

In December 1970, The Inter-Society Commission for Heart Disease Resources, "comprised of outstanding leaders in the field of cardiovascular diseases and representatives of national professional organizations capable of making significant contributions," reported its suggestions for the prevention of atherosclerotic diseases.

Dr. Oster is Chief of Cardiology, Park City Hospital, Bridgeport, Conn. The author gratefully acknowledges the assistance of Michael A. Berger in the mathematical analysis of the statistical data in the Report. Mr. Berger, a student at the University of Bridgeport, was supported in this work by a research grant from the Greater Bridgeport Heart Association.

*"Primary Prevention of the Atherosclerotic Diseases": Report of the Inter-Society Commission for Heart Disease Resources, Circulation *42*:A-55, 1970

The Commission was created through a contract with Regional Medical Programs Service to help fulfill the requirements of Section 907 of Public Law 89-239, which established the Regional Medical Programs in 1965. The Commission worked under contract No. NH 69-29 with the Health, Science, and Mental Health Administration Department of the Department of Health, Education and Welfare. The funds came out of taxpayers' money.

In its introductory note to the reader, the Commission invites constructive criticism of the work of its study groups, particularly those who attempt to apply these guidelines. In the following paper such constructive criticism will be given and it will be shown that the Report of the Commission is prejudicial, based on dubious data and therefore probably deceptive. Furthermore, by thus wasting the taxpayers' money, the Report of the Commission, instead of advancing the cause of coronary heart disease prevention, channels the research funds overwhelmingly into narrow, preconceived, subjective, discredited areas of research. It thereby hinders, if it does not preclude, the development of other approaches which may provide more effective solutions of this problem, so vital to the health of the American people.

To the tune of scheduled press conferences and through recourse to the various media, the American people were advised to change their diets drastically and especially curtail the consumption of cholesterol to 300 mgs. daily from the usual 600 mgs. and reduce the intake of saturated fats to 35% from 40%. Admittingly having no proof on which to base these revolutionary recommendations that

would affect the life habits of the American people and alter the manufacturing of basic foods and the raising of livestock, the Commission presents some alleged facts, studies, and tables to support their unproven theories.

On page A-83 of the Commission's Report[1] we find Table 16, The Estimated Relative Reduction in Coronary Heart Disease Incidence Associated with Relative Reduction in Serum Cholesterol (renumbered as Table I of this article, below). The values of "v" were obtained from the relationship $v = 1 - (1 - u)^{2.66}$ described in a study of Cornfield,[2] where "v" is the relative incidence of CHD and "u" is the relative serum cholesterol level. An example is given that a 10% reduction in the serum cholesterol level in the United States population ($u = .10$) is estimated to yield a 24.4% decrease in coronary heart disease incidence ($v = .244$).

TABLE I

ESTIMATED RELATIVE REDUCTION IN CORONARY HEART DISEASE INCIDENCE (v) ASSOCIATED WITH RELATIVE REDUCTION IN SERUM CHOLESTEROL (u)

Relative decrease in serum cholesterol (u)	Relative decrease in coronary heart disease incidence (v)	Relative decrease in serum cholesterol (u)	Relative decrease in coronary heart disease incidence (v)
.01	.026	.26	.551
.02	.052	.27	.567
.03	.078	.28	.583
.04	.103	.29	.598
.05	.128	.30	.613
.06	.152	.31	.627
.07	.176	.32	.642
.08	.199	.33	.655
.09	.222	.34	.669
.10	.244	.35	.682
.11	.267	.36	.695
.12	.288	.37	.707
.13	.310	.38	.720
.14	.330	.39	.731
.15	.351	.40	.743
.16	.371	.41	.754
.17	.391	.42	.765
.18	.410	.43	.776
.19	.429	.44	.786
.20	.448	.45	.796
.21	.466	.46	.806
.22	.484	.47	.815
.23	.501	.48	.824
.24	.518	.49	.833
.25	.535	.50	.842

Note: Values of v were obtained from relationship, $v = 1 - (1 - u)^{2.66}$ described in Fed. Proc. 21 (Suppl. No. 4):58–61, July–August, 1962.

These data illustrate the potential for prevention, and the possibility of achieving declines in CHD morbidity and mortality. For example, a ten per cent reduction in serum cholesterol level of the U.S. population ($u = .10$) is estimated to yield a 24.4 per cent decrease in CHD incidence ($v = .244$).

Since such pivotal importance is ascribed to this table and since it is utilized in two prior studies,[3,4] we deemed it worthwhile to re-read Cornfield's original work and find out if the statements contained in there correspond with the table published by the Inter-Society Commission. In the entire study of Cornfield, we were unable to find the equation $v = 1 - (1 - u)^{2.66}$ but we do find equation (7), $P = .0091\left(\frac{Y_1}{100}\right)^{2.66}\left(\frac{Y_2 - 75}{100}\right)^{1.47}$ where Y_1 and Y_2 are serum cholesterol and systolic blood pressure respectively.

Cornfield states, "A 1% decrease in cholesterol would be associated with a 2.66% decrease in risk of coronary heart disease throughout the range of cholesterol values." In other words, it is not just limited to the levels of frank hypercholesterolemia, but also applicable to the lower values of cholesterol in the serum without exception. So, if to drive this point home by *reductio ad absurdum,* a lowering of cholesterol level from a normal of 220 mgs to 110 mgs/per cent would put a theoretical person at an 84.2% less risk of coronary heart disease; the same as if lowering the cholesterol from 480 mgs. to 240 mgs/per cent, providing that the experimental reduction of cholesterol is of the same risk (a condition that the Commission fails to mention).

Cornfield states in a personal letter: "My paper, 'Joint Dependence of Risks of Coronary Heart Disease on Serum Cholesterol and Systolic Blood Pressure: a Discriminant Function Analysis' (Fed. Proc. 21, Suppl. No. 4, pp 58-61, July–August, 1962) states, 'If one could lower cholesterol levels by a given amount, say 15%, and if equation (7) describes not only the association between risk and cholesterol level in Framingham, but also the change in risk that would accompany an experimental alteration in serum cholesterol, then the relative risk would be $(.85)^{2.66}$, or a reduction of about 35%.'" Mathematical analysis reveals Cornfield's formula correct insofar as statistical significance is concerned.

Having shown the biological impracticability of the Report's Table 16, we turn to the collection of data for Cornfield's equation. We question this data, which was given to him by the Framingham Study, and also the paucity of numbers from which

TABLE II[2]

NEW CHD IN 6 YEARS FOLLOW-UP: ACTUAL AND EXPECTED NUMBER OF CASES AMONG MEN 40–59

Serum Cholesterol mg/100 cc.	Systolic B.P. mm Hg.	NEW CHD		Population at Risk
		Expected*	Actual	
200	Total	10.5	12	319
	<117	0.8	2	53
	117–126	1.4		66
	127–136	1.8	2	59
	137–146	2.3	1	65
	147–156	1.6	2	37
	157–166	0.7	1	13
	167–186	1.4	3	21
	187+ over	0.5	1	5
200–209	Total	5.9	2	133
	<117	0.5		21
	117–126	0.8	2	27
	127–136	1.4		34
	137–146	1.0		19
	147–156	1.0		16
	157–166	0.7		10
	167–186	0.4		5
	187+ over	0.1		1
210–219	Total	6.9	6	121
	<117	0.4		15
	117–126	0.9	1	25
	127–136	1.0	2	21
	137–146	1.5		26
	147–156	0.4		6
	157–166	0.9		11
	167–186	1.1		11
	187+ over	0.8	3	6
220–244	Total	22.3	23	334
	<117	0.6		20
	117–126	2.9	8	69
	127–136	4.7	2	83
	137–146	5.5	6	81
	147–156	2.4	3	29
	157–166	1.5	1	15
	167–186	3.1	2	27
	187+ over	1.6	1	10
245–259	Total	11.6	8	136
	<117	0.5		14
	117–126	1.3		24
	127–136	2.3		33
	137–146	2.0	3	23
	147–156	1.9	2	19
	157–166	1.3		11
	167–186	0.7	2	5
	187+ over	1.5	1	7
260–284	Total	16.0	23	156
	<117	1.0	1	22
	117–126	1.4	5	22
	127–136	2.1	2	26
	137–146	3.5	2	34
	147–156	1.9	4	16
	157–166	1.7	2	13
	167–186	2.7	6	16
	187+ over	1.5	1	7
285+ over	Total	18.9	18	130
	<117	0.7		11
	117–126	1.7	1	19
	127–136	3.1	4	28
	137–146	3.1	4	23
	147–156	2.8	1	16
	157–166	2.5	4	12
	167–186	3.2	3	14
	187+ over	1.9	1	7

*Expected number of cases $= \frac{\text{(population at risk)}}{[1 + e]^{(23.13 - 6.14X_1 - 3.29X_2)}}$

Note: Cholesterol determinations were done by the Kendall-Abel method at Framingham, Massachusetts

he derived his equation. We doubt the validity of the application of Cornfield's equation to Table 16 in the Inter-Society Report in view of the latter's simplifications and the omission of the risk of experimental alteration in serum cholesterol levels which Cornfield mentions. According to the Commission's attitude, this risk would be zero. The Commission implies that the reduction mentioned in the footnote to Table 16 would only be valid for the very high levels of serum cholesterol found in hypercholesterolemia, but Cornfield claims his formula is valid for the whole range of cholesterol levels.

A total of 1329 male persons between the ages of 40 to 59 were examined for freedom of coronary heart disease in the Framginham Study. Neither then (1962) nor at the present time can even the most astute diagnostician say with certainty that an individual is free of coronary heart disease. So one of the basic assumptions of the Framingham Study is open to serious doubt.

Of the 1329 allegedly coronary heart disease-free persons only 92 developed various incidents of coronary heart disease, which must have included angina pectoris. Seventy-four were distributed over a range of cholesterol from 200 to 284 mgs/per cent, with groupings as high as 23 to a low of 2 persons. The groupings were arbitrarily chosen as shown in Table II reprinted from Cornfield's paper (see at left). A questionable transformation takes place by semantic legerdemain on the part of the Commission. The *risk* of coronary heart disease in 92 persons out of a group of 1329 reported by Cornfield is equated by the Commission to the *incidence* of coronary heart disease in many millions of the U.S., if not the world's, population — a substitution which is unacceptable. Again, we doubt the accuracy of the data presented to the computer.

It is a known fact that cholesterol in the nonfasting individual, as tested in Framingham, is not greatly affected by dietary intake of fats and cholesterol in the short run. From values of our own study, described below, as well as from the world literature we learn that the same individual has variable values of serum cholesterol on different days, different seasons and other circumstances.[5]

Cholesterol was determined in five males and five females of an age group from 18 to 30 on two different occasions. On one occasion a low caloric breakfast was served consisting of two ounces of Ovaltine®* dissolved in twelve ounces of milk.

*Ovaltine® contains protein (10%), easily emulsified fat (4%), and carbohydrates (78%). Each teaspoonful contains 22 calories.

TABLE III

SCATTERED VALUES OF SERUM CHOLESTEROL IN A GROUP OF INDIVIDUALS AT VARIOUS TIMES

Key:

○ = Low caloric diet, fasting

● = Low caloric diet, post-prandial

□ = High caloric diet, fasting

■ = High caloric diet, post-prandial

Group A–G is male

Group H–N is female

Patient→ Serum Level ↓	A	B	C	D	E	F	G	H	I	J	K	L	M	N
150–159										■				
160–169		■												
170–179				●	□					□	□ ■			
180–189			□		○ ■ ●	□				●				□
190–199		○ ●	■	○				□ ■		○	○			■
200–209			●	■		■					●		□ ■	
210–219		□	○	□			■	○ ●						
220–244	■						□		○ ■ ●			○ □ ■		
245–259	●								□			●		
260–284	○ □													

Note: Accuracy of determinations, by the Libermann-Burchardt method, was ±3.5%.

On a subsequent day a high caloric breakfast consisting of bacon, eggs, sausages, bread, butter, and orange juice was consumed. Post-prandial blood specimens were examined 45 minutes after the individual finished eating the low caloric meal. Ninety minutes after the high caloric meal post-prandial blood specimens were examined. Four individuals (2 males and 2 females) aged between 30 to 62 consumed the high caloric meal with an addition of 2 ounces of Ovaltine® in 12 ounces of milk. Fasting and 90 minutes post prandial specimens were examined. There was a total of 48 different cholesterol determinations.

It was found that only eight times did the fasting and post-prandial specimens pair (see Table III, above). The other pairs of fasting and post-prandial values varied significantly in the same individual and projecting this finding to the Framingham Study, 66% of the post-prandial values used in this population group might be subject to grouping changes—a factor that would throw the application of Cornfield's equation to the Framingham Study into serious doubt.

The Framingham blood specimens were taken when convenient to the volunteers regardless of diet, time of the day, or other anamnestic influences. The accuracy of our determinations was ±3.5% as determined by duplicates. If hypercholesterolemia is defined as serum values over 260 mgs/per cent, only one individual had such a fasting serum value, and that went down 20-40 mgs/per cent post-prandially, jumping two of Cornfield's sample groups. It is also quite presumptuous to project or generalize findings found in a homogeneous northeastern community whose racial and ethnic background is not given (and which could have completely different eating habits) to embrace the considerable diversity of the United States population. It has been reported by others in epidemiological studies that the death rate from coronary heart disease is different in various states of the Union.[6]

Due to the narrow range chosen by Cornfield in his groupings, an individual's cholesterol level could shift from one of Cornfield's cholesterol groups into another, from day to day, and week to week. This would also apply to blood pressure measurements. These shifts would then raise havoc with Cornfield's formula. Again we find Cornfield's formula pitiably wanting (biologically) though statistically valid, assuming the constancy of starting values.

Cornfield also stresses the fact that the percentage reduction of cholesterol pertains to risk of coronary heart disease in all values of the table high or low. But it is different if one considers the presentation of risk in actual milligram/per cent reduction. There we have a large absolute difference in risk when added to a large, rather than to a small, initial level of serum cholesterol.

With complete disregard of the above considerations, the Commission devised its own interpretation of Cornfield's data. Instead of *risk,* we are now told that the coronary heart disease *incidence* is reduced with a relative reduction in serum cholesterol. According to Webster's Dictionary, 2nd edition, risk and incidence have two completely unrelated meanings—

Incidence: act, fact, manner of falling upon or affecting, range of occurrence or influence
Risk: degree of probability of loss or chance of loss

We are now told that a 10% reduction of cholesterol in the population of the United States will yield a decrease of 24.4% in the *incidence* of coronary heart disease. From Table 16 in the Commission's report we can learn that a 50% decrease in serum cholesterol will yield a decrease of 84.2% of the *incidence* of coronary heart disease. Any biologically oriented researcher knows that this is a physiologic impossibility and is absurd in its implication because a 50% reduction of serum cholesterol levels may entail untold dangers.

Cornfield must have sensed these shortcomings because in a later (1969) publication[7] he stated, "We must admit at the outset that we have no secure basis for estimating the reduction in incidence of or mortality from coronary heart disease that could be achieved by application of current knowledge." He also states in the same article, "It seems clear that despite a very considerable scientific effort and some tantalizingly suggestive results, we have no clear-cut generally accepted answer to the question whether cholesterol lowering measures can affect coronary heart disease." We concur in Cornfield's doubts. We feel that his equation is not valid biologically and should not be used to substantiate the implied effect of proposed dietary changes suggested by the Commission.

Obsessed with the idea of correlating values from blood chemistry with actual disease, the Commission was undisturbed by reality and objectivity. Since the Commission's mind was made up in advance, the membership of the Commission did not want to be bothered with the confusing facts. For example, in a study of cigarette smoking, the find-

ings of Keys and others[8] that there was no connection between cigarette smoking and coronary heart disease in certain European countries was not mentioned; whereas the findings concerning the relationship between cholesterol levels and coronary heart disease in the same study are given prominence. Keys also states in the same study, (again, not mentioned in the Commission's Report) that the incidence of coronary heart disease is lower among individuals who smoke less than 20 cigarettes per day than among *non-smokers* or heavy smokers. An unbiased report would not have suffered from such omissions.

In a very recent study by Keys[9] we find that in contrast to a value of 2.66 found in the Framingham Study as the exponent for coronary heart disease incidence, a value of 2.24 has now been established. There is also a quotation that angina pectoris was less closely related to serum cholesterol level than it was to myocardial infarction. This result was also duplicated by a study of Western Electric Co. employees and Los Angeles civil servants.[9] However, as mentioned before by Cornfield in his paper,[2] all phases of coronary heart disease are included in the Framingham Study. This again shows the unreliability of his original data.

Furthermore, the statements of Pearce and Dayton,[10] who warn that their findings are not to be construed as recommending a change in the American diet, are alarmingly absent in the Commission's Report. The fact that there is a potential danger from unsaturated fatty acids as carcinogenic agents is underplayed. The American people are led to believe that unsaturated fatty acids do not undergo chemical changes under heating and during cooking – though such changes are a fact well known to every organic chemist but not mentioned in the Inter-Society Commission Report.

If publications of the individual members constituting the Commission are examined, one finds that in July 1970 J. T. Doyle wrote, in an editorial in the Annals of Internal Medicine,[11] about the prevention of coronary heart disease: "The conscientious physician must then be guided by his instincts, his common sense, and the fundamental principle that he should do no harm." In December of the same year one finds him as a member of the Commission giving his consent to sweeping recommendations of dietary changes.

A more cautious view of such dietary changes is reflected in the remarks of Dr. Donald S. Frederickson when discussing the Inter-Society Commission Report in his St. Cyres Lecture of the National Heart Hospital at the Royal College of Physicians of London, published in the British Medical Journal:[12]

> "There are some things about the report to be pitied, however. In the light of what is actually known, the injunctions on consumption of cholesterol and fats seems too radical as they stand. What evidence do we have that an egg yolk a day spells jeopardy for *all* Americans?" . . .
>
> ". . . One looks vainly in the Commission Report for something new and strong enough to change the present governmental non-position concerning dietary fat consumption." . . .

The Commission disregards the potential economic dislocations which would result from their recommendations, should they become reality. It also completely disregards the warnings and excellent deliberations in the Report of the Diet-Heart Review Panel of the National Heart Institute (dated June 1969 and published as American Heart Association Monograph No. 28, 1969, which antedates the Inter-Society Commission Report). The members of that panel have at least the same scientific standing and authority, but act more responsibly than the members of the Inter-Society Commission.

By critical analysis of the Report of the Inter-Society Commission for Heart Disease Resources concerning the prevention of atherosclerosis one is led to endorse the opinions of Ludwig and Collette:[13] "Errors of presentation include improper generalizations and the incautious use of second-hand data." We feel that the Commission presented its findings in a misleading manner.

We quote again from Ludwig and Collette,[13] "More serious consequences follow when erroneous conclusions are drawn from invalid data or through faulty or incomplete analysis. Health programs may be augmented or discontinued, health practices modified, and worthless solutions to health problems advanced, all on the basis of misinformation."

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CRITIQUE:

Limitations of the Statistical Methods Used in the Framingham Study of Risk Factors in Coronary Heart Disease

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In the foregoing paper Oster takes as his starting point a paper by Cornfield, who tried to apply bivariate gaussian statistics to the relation between three parameters: serum cholesterol level, systolic blood pressure and the risk of coronary heart disease.[1]

In view of the assumptions necessary with Cornfield's approach and possible misunderstanding of their application by others, it is first necessary to review Cornfield's methods and his reason for choosing them.

The relative frequencies of those who would get coronary heart disease (CHD) and those who would not (NCHD) as functions of serum cholesterol level and systolic blood pressure are not known. It is possible to obtain these frequencies if enough observations can be made. They can often, but not always, be made in the physical sciences; they can seldom, if ever, be made in medicine.

In a later paper (not mentioned by Oster), Truett, Cornfield and Kannel[2] allude to a cohort of perhaps several hundred thousand persons required for multiple cross-classification analysis of the several risk factors involved in coronary heart disease. This contrasts with the less than 5,000 subjects reported in later Framingham studies and less than 1500 in the earlier ones.

Faced with the dilemma of an insufficient number of observations and a desire to have a decent confidence interval, Cornfield guessed the mathematical descriptions of the CHD and NCHD relative frequencies. He assumed that both relative frequencies were bivariate log-normal. His reasons for choosing these statistics were twofold: a non-rigorous agreement of his assumption with the observations as well as computational ease. Thus, instead of having to determine the entire relative frequency functions, Cornfield had only to estimate the means, variances and covariances of the observed data for the CHD and NCHD populations.

These relative frequencies cannot be log-normal because an infinite range in serum cholesterol and systolic blood pressure is implied in the first assumption. Results of this assumption which depend on the tails of these distributions should be carefully examined.

The second assumption states a relation between the CHD and NCHD relative frequencies. This assumption is that these relative frequencies have identical variances and differ only in their means. While this assumption again has a basis in observed data, its computational significance is tremendous. Bayes' theorem is applied to get the conditional probability distribution function of CHD risk given the serum cholesterol level and systolic blood pressure:

$$P(CHD \mid Y_1, Y_2) = \frac{1}{1 + \dfrac{P(NCHD)\, p(Y_1, Y_2 \mid NCHD)}{P(CHD)\, p(Y_1, Y_2 \mid CHD)}} \quad (1),$$

where:

Y_1 = serum cholesterol level in mg/per cent.
Y_2 = systolic blood pressure in mm. of Hg.
$P(CHD \mid Y_1, Y_2)$ = conditional probability distribution function of CHD risk given Y_1 and Y_2
$P(NCHD)$ = probability of NCHD
$P(CHD)$ = probability of CHD
$p(Y_1, Y_2 \mid NCHD)$ = conditional probability density function (relative frequency) of Y_1 and Y_2 given NCHD
$p(Y_1, Y_2 \mid CHD)$ = conditional probability density function (relative frequency) of Y_1 and Y_2 given CHD.

This expression, which is equivalent to Cornfield's equation (1), shows that the relative frequencies appear as a ratio. The second assumption means that the exponent of the gaussian exponential must be a linear function — namely equation (1) reduces to:

$$P(CHD \mid X_1, X_2) = \frac{1}{1 + e^{-(\alpha + \beta_1 X_1 + \beta_2 X_2)}} \quad (2)$$

where:

$X_1 = \log_{10}(Y_1/100)$
$X_2 = \log_{10}((Y_2 - 75)/100)$
$\beta_1 = 6.14$ } computed functions of means, variances,
$\beta_2 = 3.29$ } and covariances of the relative
$\alpha = -23.13$ } frequencies.

This expression is equivalent to Cornfield's equation (5).

In an effort to make equation (2) still more attractive computationally, Cornfield made a third assumption: that $P(CHD \mid X_1, X_2)$ was small, so that

$$P(CHD \mid X_1, X_2) \approx \frac{P(CHD \mid X_1, X_2)}{1 - P(CHD \mid X_1, X_2)} \qquad (3).$$

Equation (3) states that .25 equals .2 for P equal to .2, for an error of 25%. For P equal to .1, we have .11 equal to .1 for an error of 10%. By restricting his study to small risk cases, Cornfield made use of this approximation. Substituting equation (3) into equation (2), Cornfield got:

$$P(CHD \mid X_1, X_2) = (e^{\alpha})(e^{\beta_1 X_1})(e^{\beta_2 X_2}) \qquad (4).$$

Substituting in the values for X_1, X_2, β_1, β_2 and α, Cornfield then got as his equation (7):

$$P(CHD \mid Y_1, Y_2) = .0091\left(\frac{Y_1}{100}\right)^{2.66}\left(\frac{Y_2 - 75}{100}\right)^{1.47} \qquad (5).$$

Oster complains of the absence of the equation $v = 1 - (1 - u)^{2.66}$ in Cornfield's paper, "Joint dependence of risk of coronary heart disease on serum cholesterol and systolic blood pressure: a discriminant function analysis," which equation is mentioned in the Inter-Society Commission's Report (Table 16, repeated as Table 1 in Oster's paper). That equation is derived as follows:

In order to compare the risk P′ relative to P at a serum cholesterol level of Y_1' relative to Y_1 while keeping the *same* systolic blood pressure, we divide both applications of equation (5) getting:

$$\frac{P'}{P} = \left(\frac{Y_1'}{Y_1}\right)^{2.66} \qquad (6).$$

Equation (6) is more conveniently expressed in terms of relative decreases. Letting $Y_1'/Y_1 = 1 - u$, and $P'/P = 1 - v$, we get:

$$v = 1 - (1 - u)^{2.66} \qquad (7).$$

This equation is subject to the correctness of Cornfield's assumptions, which were necessary to derive it. In particular, it is valid for only those values of systolic blood pressure and serum cholesterol level for which Cornfield's third assumption is valid. Figure 1. (see next page) was prepared to show the estimated reduction in the 6-year risk of CHD associated with a 10% reduction in serum cholesterol level, without making Cornfield's third assumption. Oster correctly noted the absurdity of the medical implications encountered when taking equation (7) literally.

In deference to Cornfield, he qualifies the use of equation (7) in a footnote:

> *"For some of the values of X_1 and X_2 observed, P considered as the 6-year risk is too large for the approximation to be entirely accurate. For the 1-year risk, which is one-sixth of the 6-year risk, the approximation holds for all observed X_1 and X_2."*

Others, including the National Diet-Heart Study Group, have not heeded his caution.

Cornfield's artifice is to look at the 1-year risk of CHD rather than the 6-year risk and assume that the incidents of CHD are uniformly distributed with time. Not only might this additional assumption not be true, but he also ignores the desire to isolate the long term factors associated with the risk of CHD.

One reason why great importance was placed on the serum cholesterol level rather than on systolic blood pressure is the fact that in equation (5) the exponent of the serum cholesterol level factor (2.66) is greater than that for systolic blood pressure (1.47). Comparing the relative importance of different physical quantities must be done in a manner not affected by the actual physical units of measure. If systolic blood pressure were measured in kilometers of mercury instead of millimeters, then its exponent would have been larger than that of serum cholesterol. Standard units (dimensionless σ-units) should have been used before a relative importance comparison had been made. In the absence of this transformation, no valid comparison between the relative importance of serum cholesterol level and of systolic blood pressure can be made from equation (5).

To illustrate the consequences of using natural units, consider the following example. It is proposed to reduce the serum cholesterol level by 10% and thereby reduce the relative risk of CHD by 24.4%, according to equation (7). Assuming that this equation has been properly used, it appears to be a big payoff for a little effort. This may not be true because the 10% reduction is in natural units. It could be that this 10% reduction represents going from the 75th percentile to the 25th percentile, quite an undertaking. Without using standard

Figure 1.

*Estimated Relative Reduction in the 6-year Risk of CHD Associated with a 10% Reduction in Serum Cholesterol Level Without Making Cornfield's Third Assumption**

Serum Cholesterol mg/100 cc	Systolic Blood Pressure, mm Hg							
	<117	117–126	127–136	137–146	147–156	157–166	167–186	⩾187
<200								
200–209								
210–219								
220–244								
245–259								
260–284								
⩾285								

Reduction between 23.3 and 24.4%

Reduction between 7.5 and 23.2%

Reduction less than 7.5%

**Based on the 1962 paper of Cornfield.[1] It is interesting to note that in the 1967 paper by Cornfield[2] (and others) he no longer discusses estimated relative reductions in the risk of CHD, though dealing there with seven risk factors, as opposed to two in the earlier paper.*

units, there is no way of gauging how difficult it is to achieve a percentage reduction. In a later paper,[2] Cornfield uses standard units.

In Figure 1, I have used the same groupings of serum cholesterol level and systolic blood pressure which are used by both Cornfield and Oster. Care must be taken to ask correct questions when using statistical methods. Predictions cannot be made of the risk of CHD for people having a serum cholesterol level of exactly 220 mg/per cent. Indeed, the answer to this question is another question: what is meant by 'exactly' 220 mg/per cent? Oster's own experiment involving serum cholesterol level measurements had a measurement accuracy of 3.5%. Thus by 'exactly' 220 mg/per cent, Oster could mean any measurement from 211 to 227 mg/per cent. The same analogy applies to statistics. The risk of CHD can be predicted from people having a serum cholesterol level falling within a range from a to b. Cornfield's choices of the ranges for both serum cholesterol level and systolic blood pressure are his own. *These ranges do not constitute discrete thresholds for risk.* Cornfield states that the risk of CHD varies gradually with both serum cholesterol level and systolic blood pressure. This behavior is a direct consequence of the assumption of gaussian statistics.

Oster states that his experiments show that fasting and post-prandial serum cholesterol levels may not fall within the same or even adjacent ranges. He further states that this factor "would throw the application of Cornfield's equation to the Framingham study into serious doubt." This is a dubious statement because whether or not the serum cholesterol level falls within one adjacent range or another is not that critical.

Oster however, has missed an important point: what might the Framingham results have been like had the measurements been taken in a uniform manner? In a later paper,[3] Cornfield states that it is not possible to show statistical significance between the risk of CHD and serum cholesterol levels because of the large variance of this parameter. One reason why this variance may have been so high might have been the manner in which these measurements were taken. Indeed, if these measure-

ments were taken in a uniform manner, then incontrovertible statistical significance might have resulted.

Oster criticizes the Framingham study for using only 1329 subjects. I doubt if this sample size was limited by design. In a later study by Truett, Cornfield and Kannel[3] 4856 subjects were used. Interestingly, Oster is ready to challenge the validity of the Framingham study on the basis of his own experiments, which had a total of only 48 cholesterol determinations, prior proof notwithstanding.

According to the National Diet-Heart Study Final Report,[4] the effect of reducing the daily consumption of cholesterol by 300 mg/day and of reducing the consumption of saturated fats to 35% would be to reduce the serum cholesterol level by 25 mg/per cent, or about 10%. Given this 10% reduction we can make the following statements about the risk of CHD:

1] those people whose serum cholesterol level is 10% less than the *average low risk* population are 24.4% less likely to get CHD;

2] those people whose serum cholesterol level is 10% less than the *average population with 10% risk* of CHD are 23.3% less likely to get CHD;

3] those people whose serum cholesterol level is 10% less than the *average population with 20% risk* of CHD are 7.5% less likely to get CHD.

These numbers were derived by using equation (2) instead of equation (7). [Equation (7) makes use of the third assumption referred to earlier in this critique.] They show the errors which result when equation (7) is incorrectly used for the *entire* range of CHD risks, as it is in Table 16 of the Inter-Society Commission Report (renumbered Table I by Oster for use in his paper).

One must be careful to differentiate between risks for different populations and cause/effect. Statistical studies of population samples, such as the Framingham Study, cannot show *how* a reduction in an individual's serum cholesterol level would affect his risk of CHD. These studies are designed to suggest areas for further research. As a result of this initial study,[1] additional parameters were examined at Framingham.

In a 1967 paper,[2] multivariate gaussian statistics were applied to seven parameters: age; cholesterol; systolic blood pressure; relative weight; hemoglobin; number of cigarettes smoked; and ECG abnormality. This report tended to diminish the importance of cholesterol. It also discerned differences in the relative importance of these parameters as a function of age. It is unfortunate that Oster did not choose to comment on this paper in addition to the earlier one: he might have found more ammunition.

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EVALUATION OF SERUM CHOLESTEROL REDUCTION AND XANTHINE OXIDASE INHIBITION IN THE TREATMENT OF ATHEROSCLEROSIS

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From the pageant of illustrious congresses concerned with prevention of atherosclerosis has emanated a multitude of dissonant statements. The most recent opinion has been issued by the National Heart and Lung Institute Task Force on Arteriosclerosis (1971), which has stated, "Although there is some evidence to support the popular belief that blood lipids are causally related to arteriosclerosis, and that a decrease in total and saturated fats in the diet may help to prevent the complications of arteriosclerosis such as heart attack and stroke, this evidence is scientifically not entirely convincing. Therefore, recommendations concerning diet are based on personal impressions and fragmentary evidence rather than on scientific proof. . . . Convincing evidence should be sought that lowering the levels of lipids in blood reduces morbidity and mortality from arteriosclerosis."

A recent coronary drug project used dextrothyroxine to lower serum cholesterol in patients with prior myocardial infarction, in an attempt to confer protection against cardiac death or morbidity (Stamler and group, 1972). The attempt was a dismal failure. There were 18.4% more deaths among the persons treated with the cholesterol-lowering drug than in a parallel placebo study, despite the fact that their cholesterol values were about 10% lower than base levels. According to Cornfield's (1962) formula, $V = 1-(1-u)^{2.26}$, a 24.4% relative reduction in coronary heart disease incidence (V) is associated with a 10% relative reduction of serum cholesterol (u). The observed 18.4% death increase with dextrothyroxine should then be added to the expected 24.4% reduction, resulting in a disappointing 42.8% increase of coronary heart disease incidence.

The Veterans Administration Drug Lipid Cooperative Study Group (Schoch, 1972) also reported a failure of cholesterol-lowering drugs to influence coronary mortality in a double blind 5-year secondary prevention study. Drugs used were estrogen, estrogen combined with dextrothyroxine, estrogen combined with aluminum nicotinate, and aluminum nicotinate alone.

In evaluating a cholesterol-reducing trial using clofibrate, Friedewald and Halperin (1972) observed that the cholesterol-lowering effect of the drug was actually greater among those subjects who died from cardiac causes than among

those who survived. It should be reiterated that the effectiveness of a drug does not always coincide with the intended rationale of its use.

Keys *et al.* (1972), in a 5-year probability study of developing coronary heart disease in middle-aged men, compared the influence of such variables as age, blood pressure, serum cholesterol, and smoking habits. They concluded that incidence of coronary heart disease is strongly influenced by one or more variables unrelated to any of those considered, since the U.S. men in the study had an incidence rate of hard coronary heart disease approximately double that of European men with the same "risk factors."

One of the puzzling factors in most population studies is the certainty with which investigators can diagnose subjects as "free of coronary heart disease" at the outset. Even such language modification as "free of evidence of coronary heart disease" or "judged to be free of coronary heart disease" does not hide the fact that there is almost no knowledge of that part of the "iceberg of atherosclerosis" (Katz, 1972) which lies below the surface of recognizable identification. One should admire the perspicacity of the certifying physicians who declared 8,728 European men and 2,404 U.S. railroad men (and a four-pool total of 6,221 U.S. men) aged 40 to 59 years to be free of coronary heart disease. Even with the most refined techniques available in medical centers over the world, this is not yet possible in 1972, much less at the time some of these studies were started–1948 and 1953. It would probably be more honest and pertinent to state that unknown numbers of atherosclerotic lesions and unknown degrees of incipient coronary heart disease are present in North American men after the age of 20, or, to coin a phrase, "every man and woman in the United States over 20 years of age has atherosclerosis until proven otherwise."

Since there seems to be no established causal relationship between so-called risk factors and the presence of atherosclerosis and coronary heart disease in human beings living under different geographical conditions, and since there appears to be good evidence that a dietary factor is the cause of early atherosclerosis, one feels compelled to seek a difference in the dietary makeup of various population groups. It is expected that this theoretical dietary factor would behave like a naturally occurring toxicant and would be found not only in food but also in the tissues of the human body. One would then deal with toxic effects rather than with deficiency effects. It is thus difficult to understand the application of animal experimentation with foods toxic for the particular species, with resultant changes in many tissues and organs of the affected animal, to the human pathology of atherosclerosis, which allegedly affects only the arterial system.

Most toxic substances of plant and animal origin have been found by trial and error; many of these have been recognized by developing civilized man since antiquity. Some religious dietary taboos have their origins in this intuitive knowledge. The mode of action of these substances differs in many species, depending mainly on absorption. Many food toxicants act as antimetabolites,

some destroy essential vitamins, and some contain too much of a potent vitamin, *e.g.*, vitamin A (Mickelsen and Yang, 1966).

Arterial wall changes leading to atherosclerosis have been attributed to the enzyme xanthine oxidase contained in bovine milk (Oster, 1968, 1971). When absorbed, this enzyme may act as a food toxicant. Xanthine oxidase, aided by phospholipases, can oxidize the fatty aldehydes contained in the phospholipid plasmalogen into their respective fatty acids. This chemical reaction creates a cell membrane injury which needs repair. The repair mechanism varies in different anatomical locations. In the arterial wall, following possible initial cell proliferation, repair would be accomplished by cholesterol ester infiltration and fibrin deposition. In heart muscle cells an influx of scar tissue would occur. The pathological process involved has been called "plasmalogen disease" by Oster (1971).

There is a disturbing unknown in this theory, namely, the question of how the large xanthine oxidase molecule (molecular weight about 290,000) passes through the intestinal wall. As yet there is no direct proof of this phenomenon in humans. There are, however, direct and indirect clues that intact protein molecules can be absorbed. Clark (1959) proved the absorption of proteins and colloidal material by columnar epithelium of the small intestine of suckling rats and mice. Davies (1969, 1971) demonstrated that antibodies to cow's milk protein are significantly increased in the blood of male patients with ischemic heart disease.

Osborn (1968) studied the coronary arteries of 109 children and found that most of those with more than 2 months of breast feeding were normal, while those with no breast feeding were mainly abnormal. He opined that the ill effects of artificial feeding are due to casein curds. Gunther *et al.* (1960, 1962), using the coated tanned red cell technique to measure serum antibody levels of normal infants to cow's milk proteins, found high antibody titers in the sera of 286 infants aged 7 to 97 weeks. These antibodies were specific for casein, α-lactalbumin, and bovine plasma albumin, but none was found for β-lactoglobulin or human milk proteins. Annand (1972) believes that heated (pasteurized) milk protein may have thrombogenic properties.

More and Haust (1968) found coronary arteries of 20 humans, ranging in age from the neonatal period to 25 years, which contained atherosclerotic lesions apparently superimposed upon well developed, rather than thin, intimas. It is interesting to note that the incidence of atherosclerotic complications is significantly reduced in population groups with a high degree of lactose intolerance (Tejada *et al.*, 1968).

Considering these findings, one may assume that some milk proteins escape the action of peptidases and other proteolytic enzymes and may be absorbed, probably by the intestinal lacteals, through pinocytosis and carried via the chylomicrons through the lymph stream into the circulatory system.

Although the actual absorption of xanthine oxidase has not yet been demon-

strated, its ectopic position was observed in human autopsy material (Ross, Ptaczinski, and Oster, 1972). Xanthine oxidase activity was expressed as moles of 2-amino-4-hydroxy pteridine, or AHP, oxidized per gram of tissue per hour. A mean xanthine oxidase activity in apparently normal aortic tissue was determined as between 0 and 4.7% activity. Xanthine oxidase activity in the aortic plaque varied between 33.5% and 89.5%. Normal myocardium had zero activity. Diseased heart muscle showed xanthine oxidase activity of from 26.3% to 65.3%. These are significant differences. No enzyme activity could be demonstrated in the hearts of patients who died of causes other than coronary heart disease.

Knowledge of the prolonged presence of an ectopically deposited enzyme in both the heart and the arterial wall prompted a search for a suitable inhibitor of this continually active enzyme. Such an inhibitor might then be considered a therapy for atherosclerosis, as suitable preheating of milk was considered a prevention of atherosclerosis (Oster, 1971, 1972).

In a previous study, allopurinol was utilized for its xanthine oxidase-inhibiting effect (Oster, 1968). This double-blind study had as its indicator the reduction of nitroglycerin intake in a group of patients suffering with severe angina pectoris. However, inherent shortcomings became apparent. The double-blind status was difficult to maintain, because patients who responded favorably to a pain-relieving drug soon noticed when such a drug was replaced by a placebo. Relief of angina pectoris was not a sufficiently accurate criterion to test the hypothesis of plasmalogen disease, since chest pain could have originated from many other causes in addition to plasmalogen depletion. Allopurinol in some patients may elicit certain untoward effects which require constant monitoring of blood cell counts, liver function, and other parameters.

Therefore, a nontoxic compound with similar xanthine oxidase-inhibiting qualities was sought. Such a compound was found in folic acid. The xanthine oxidase-inhibiting effect of pteridine was first described in 1950 (Kalckar, Kjelgaard, and Klenow); this effect has since been reconfirmed *in vitro*.

These studies contributed to the theory that effective doses of folic acid might inhibit sufficient xanthine oxidase to demonstrate an *in vivo* suppression of hyperuricemia. Subsequent tests with suitable patient material were not double-blind and were conducted with a changing dose level to demonstrate a dose-response dependency. Some of the results are summarized in table I.

Analysis of the data shows an almost uniform decrease of elevated uric acid levels with varying doses of folic acid. It appeared that daily folic acid therapy for a minimum of 9 days was necessary to reduce serum uric acid levels to normal threshold values. Each case was treated on an individual basis, since the cause of hyperuricemia may vary in different individuals. No change in thiazide medication, which frequently causes hyperuricemia, was undertaken with the institution of folic acid therapy. Folic acid did not increase the urinary excretion of uric acid.

Table I

Effect of Folic Acid on Serum Uric Acid
(mg per 100 ml) Serum Uric Acid

Sex & Age	Starting Average	9-21 Days' Treatment	Reduction
69, M	13.9	4.5	9.4
63, M	8.7	6.0	2.7
72, M	10.1	5.2	4.9
39, M	10.6	7.3	3.0
62, F	10.9	5.6	5.3
74, F	7.5	7.5	0
72, F	8.0	6.6	1.4
63, F	10.0	8.5	1.5
58, F	8.5	5.0	3.5
65, M	9.8	6.2	3.6
70, F	10.6	6.8	3.8
77, F	8.0	7.7	0.3
52, M	7.8	6.3	1.5
48, M	7.5	5.8	1.7
65, F	8.1	6.9	1.2
Average	9.4	6.45	2.95

It was found that if a patient halted the folic acid regimen, a follow-up examination disclosed a rise of the serum uric acid almost to the pretreatment level. Unfortunately, these observations did not reveal the source of the xanthine oxidase which was inhibited, the enzyme situated normally in the liver and small intestine, or the one ectopically deposited in the arterial wall, or both. Since it is known that active ectopic enzyme has been found in older persons, it was hoped that the ectopic enzyme was inhibited by folic acid and that liver damage would be prevented.

DISCUSSION

The all-pervasive cult of citing serum cholesterol as a significant factor in the genesis of coronary heart disease and the careless use of such nonapplicable terms as coronary thrombosis, occlusion, and myocardial infarction lead to artefactual animal experiments not pertinent to the human pathophysiological counterpart. It is amazing that in applying the hyperlipidemia theory little use is made of an important group of compounds in the lipid family, the phospholipids. In one of the most frequently quoted review articles on fat transport, Fredrickson, Levy, and Lees (1967) wrote, "One cannot escape the intuitive conclusion, however, that the phospholipids, always predictable in their composition and varying only sluggishly in concentration, are mainly in plasma to function as 'biologic detergents.' "

It is difficult to argue with "intuitive conclusions," just as it is difficult to assess the prudence of some diets. It has been demonstrated that an essential phospholipid, plasmalogen, undergoes significant changes in its relationship with the other phospholipids (lecithin, sphingomyelin) which comprise the cell membrane (Buddecke and Andresen, 1959). The diminution of plasmalogen has been ascribed to the enzymatic action of phospholipases and of xanthine oxidase. The latter may be absorbed from ingested bovine milk, a process which starts in early youth when milk consumption is high. Although this theory is not completely proven, it offers an explanation for many more facets of atherosclerosis and heart disease than does the hyperlipidemia theory. The process of xanthine oxidase absorption is not sufficiently clear, but it should be stressed that absorption of similar larger proteins has been demonstrated. Immunopharmacology might contribute to the proof that milk proteins may be absorbed and that their immune reactions are higher in atherosclerotic patients than in normal ones. A more comprehensive study than the present one is needed for confirmation.

When treating a chronic disease one searches for a modality which offers the least risk and which can be administered for long periods with no dangerous side effects or untoward reactions. Pharmacological doses of folic acid fit these conditions; its complete lack of toxicity is known.[1]

At the onset of this investigation a search was made for a nontoxic xanthine oxidase inhibitor which would facilitate proof of the theory that this enzyme is intimately involved with the genesis and continued development of atherosclerosis and certain myocardiopathies. Others, as stated in the introduction, have attempted to prove the hyperlipidemia theory by reducing serum cholesterol and studying the effect on diminished mortality from coronary heart disease. Finding a suitable model in human pathology to test prolonged pathophysiological processes is a challenge which has not been met to anyone's satisfaction. Peripheral atherosclerosis, especially prevalent in diabetic patients, in whom it causes ischemic ulceration of toes and feet, may be more suitable for short-time observation than long-range studies of coronary heart disease.

SUMMARY

Attempts to treat the sequelae of atherosclerosis with cholesterol-lowering drugs have thus far been unsuccessful; one investigator has even admitted to an increase in cardiac mortality rather than the predicted reduction. Evidence is cited that intact milk proteins are absorbed by the human intestinal tract. The newly proposed theory of the genesis of atherosclerotic lesions as caused by dietary xanthine oxidase from bovine milk is thereby indirectly supported. Data are given to show the presence of xanthine oxidase in myocardial tissues of patients with coronary heart disease. Treatment of xanthine oxidase-induced plasmalogen diseases would consist of enzyme inhibitor not toxic in large doses and prolonged administration. Folic acid is suggested as the modality having the desired pharmacological properties and *in vivo* effects of reduced hyperuricemia.

[1] Ongoing studies in this direction were seriously hampered when in autumn, 1971, the U.S. Food and Drug Administration prohibited the dispensing of folic acid in individual doses larger than 1 mg. Larger dosages had been available since 1947.

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...ents with acute pancreatitis, however, they found that the ...nd the ratio are uniquely increased, the latter to 6.6 ± 0.3 per ... Dr. Warshaw of the Massachusetts General Hospital has ... the values to be 3.1 ± 0.1 per cent and 10.2 ± 4.2 per cent in ... persons and patients with pancreatitis, respectively.)

... Powell did indeed obtain a result in the pancreatitis range but ...s by virtue of an error both arithmetic and conceptual — that ...essing serum creatinine in terms of mg per 100 ml and the ...ariables in terms of U or mg per milliliter, which effectively ...ed the ratio by 100. When the actual units are used, the re... 0.0841.

$$\frac{222\ \text{U/ml}}{56\ \text{U/ml}} \Big/ \frac{0.66\ \text{mg/ml}}{1.4\ \text{mg/100 ml}} = \frac{8.41}{100} = 0.0841.$$

...his, unfortunately, one may have acquired the notion that ... greater than C_{Cr} and, equivalently, failed to realize that Dr. ...s result is really expressed in per cent. Moreover, unless con... dimensions are employed and the ratio multiplied by 100, it ... be used clinically with success.

...Levitt and his colleagues demonstrated, the ratio is particular...ul in detecting macroamylasemia (0.34 ± 0.13 per cent), in ...alizing" the hyperamylasemia resulting from renal dysfunc... even of severe degree (2.1 ± 0.2 per cent) and in detecting pan...s and following its course in individual patients. Neither they ... Powell, however, provided information regarding the values ...ratio in patients with hyperamylasemia owing to the other im... diagnostic considerations suggested by Dr. Powell in his dis...

Richard I. Levine, M.D.
...e, Cal. Orange County Medical Center

PLASMALOGENS

...he Editor: It is difficult to understand why Drs. Jackson and ... should have neglected to include in their review of phospho... in biology and medicine (N Engl J Med 290:24, 87, 1974) ... extensive research on phosphatidylcholine, phosphatidyl... and phosphatidylethanolamine, commonly called plasmalo... It is an established fact that about 30 per cent of the phospho... in human myocardium are plasmalogens. Studies have shown ...he disappearance of plasmalogens, essential components of the ...embrane, from the arterial wall and the myocardium, is associ...with the incipient atherosclerotic process and the onset of myo...al infarction.[1,2] These changes are independent of aging. The ... increase of sphingomyelin referred to in the review could ... ascribed to this disappearance of plasmalogen from estab...sites as well as disappearance of phosphatidylcholine and ...atidylethanolamine.

...h a process was explained as resulting from the influence of an ... derived from bovine milk, xanthine oxidase (E.C.1.2.3.2), on ...al (palmitaldehyde), the aldehydic moiety of plasmalogen.[3] ...esence of xanthine oxidase in atherosclerotic lesions has been ...ed.[4] Subsequent cholesterol-ester accumulation would then ...mpensatory repair and not a causative mechanism. Jackson ...tto present an alternative hypothesis for plaque formation, ...ting an initial net-increase in the arterial concentration of ...holipids rich in saturated fatty acids. They also regard the ac...lation of cholesterol as a secondary phenomenon. I am at a loss, ...er, to ascertain why there should be an initial increase in the ...al concentration of phospholipids rich in saturated fatty acids, ...most researchers feel that there is a proliferation of arterial ... muscle cells caused by damage to the endothelial wall from a ...al insudative process. Bovine xanthine oxidase absorbed from ...ary source may well be considered a factor contributing to this ...inflammatory process associated specifically with plasmalo...pletion.

...ertheless, despite its omission of plasmalogens, Jackson and ...s review article of 1974 is a considerable improvement over a ...view of phospholipids in the *Journal,* which stated, ". . . one ... escape the *intuitive conclusion* [emphasis mine] that the phos...ds, always predictable in their composition and varying only sluggishly in concentration are mainly in plasma to function as 'biologic detergents'."[6]

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The above letter was referred to the authors of the article in question, who offer the following reply:

To the Editor: Exclusion of the plasmalogens in our review article on "Phospholipids in Biology and Medicine" was due to the fact that we could find no studies to implicate them in membrane enzyme function or in lipoprotein structure. Despite a relative ignorance of the functional importance of this class of phospholipids, perhaps some comment about them should have been made, particularly regarding their contribution to the choline phospholipids of the myocardium.

Dr. Oster calls attention to his theory that plasmalogens are involved in atherosclerosis in the following way: (1) xanthine oxidase from homogenized bovine milk is absorbed from the gut and is eventually deposited within the arterial wall; (2) plasmalogens within the arterial wall are broken down to form the plasmal or palmitaldehyde, and (3) the palmitaldehyde is oxidized through the influence of xanthine oxidase to form palmitic acid, which contributes to an inflammatory process and tissue destruction. In our opinion, this theory is highly implausible. Xanthine oxidase is irreversibly denatured at pH 3. At the pH of the stomach, flavin, iron and molybdenum would be cleaved from the enzyme, thus inactivating it. Since the subunit weight of this protein is 150,000, it is most unlikely that bovine xanthine oxidase would pass the intestinal barrier and reach the circulation in an active form. The theory is further weakened by the fact that the postulated cleavage of the plasmalogen to form plasmal is an acid-catalyzed elimination requiring approximately pH 1. If such a reaction were to occur in the arterial wall and the corresponding oxidized products were removed from the wall, it is not clear why cholesteryl ester should replace the plasmalogen.

Finally, it should be mentioned that our hypothesis for initiating plaque formation was based on new information concerning the role of phospholipids in membranes. Although we agree that endothelial-wall damage is important in the pathogenesis of atherosclerosis, our hypothesis would require that cell proliferation be a secondary process that resulted from changes of membrane phospholipids and cholesterol that affect various enzyme systems.

Antonio M. Gotto, Jr., M.D.
Richard L. Jackson, Ph.D.
Houston, Tex. Baylor College of Medicine

CHEMOTACTIC-FACTOR INACTIVATOR IN HODGKIN'S DISEASE

To the Editor: The report that there are elevated serum levels of chemotactic-factor inactivator (CFI) in patients with Hodgkin's disease is an exciting discovery (N Engl J Med 290:76-80, 1974). The authors state that they have been unable to develop antibodies to

April 17, 1975

Mrs. Mary Winston
American Heart Association
44 East 23rd Street
New York, New York 10010

Dear Mayy:

Thanks for letting me see the latest materials re Oster! Several of these had not come to my attention. Most important is the letter to the editors of the New England Journal by Tony Gotto.

Sincerely,

Robert E. Shank, M.D.

RES/sm

enclosures