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NUTRITION RESEARCH FROM RESPIRATION AND VITAMINS TO CHOLESTEROL AND ATHEROSCLEROSIS

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The invitation to prepare this review mentioned that "prefatory chapters are more personal and philosophical than the usual critical reviews in ANNUAL REVIEWS." As the title of this review suggests, I shall recount some of the research with which I have been associated during the past 60 years.

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THE EARLY YEARS

My research activities began in my senior year at the University of Wisconsin in September 1930. My advisor, C. A. Elvehjem, assigned me to study the phosphorus partition in the blood of rachitic and nonrachitic calves as a part of an ongoing cooperative study between the Departments of Agricultural Chemistry and Animal Husbandry. This research became the basis for my senior thesis and resulted in my first publication (36).

In the fall of 1930, Dr. Elvehjem returned from a year as a postdoctoral fellow in the laboratory of Sir Frederick Gowland Hopkins, chairman of the Department of Biochemistry, University of Cambridge, England, and brought with him 12 Warburg manometers which were not then available in this country. In addition to studying the partition of phosphorus in blood, I learned how to use these manometers to study tissue respiration. These studies resulted in a publication (37) in which we reported the oxygen uptake for various tissues of chicks and rats under different nutritional conditions. In normal chicks, the rates of oxygen uptake for liver, kidney, and brain tissue are all in the range of 1000 to 1500 μL per gram of fresh material per hour. Muscle, both cardiac and striated, has a considerably lower oxygen requirement with values ranging from 230 to 380 μL. With the exception of the cerebellum, the oxygen uptake of tissues from chicks deficient in vitamins A and B_1 showed little difference from tissues of normal chicks. We found no significant difference in the uptake of liver tissue from normal and anemic rats.

Other studies of tissue respiration in which I was involved as a graduate student concerned the relation of copper to tissue respiration and the effect of certain reducing substances in chronic fluorosis and scurvy in the guinea pig (29). The research on fluorosis and the use of sodium fluoride as an enzyme inhibitor in many of these respiration studies interested me because, at about the same time, others had found that fluoride in small concentrations in drinking water was markedly effective in reducing dental caries in children. Thus began my long interest in the fluoridation of water.

In 1934, I was awarded a PhD degree at the University of Wisconsin. My thesis, only 35 double-spaced, typewritten pages, was titled "Studies on the Respiration of Animal Tissues." A few months before obtaining my PhD, I received a postdoctoral fellowship from the Rockefeller Foundation and spent the following year in the laboratory of Philip Shaffer, chairman of the Department of Biochemistry, Washington University, St. Louis. With Shaffer, I studied hepatoflavin, thought to be a portion of what was then called the vitamin B_2 complex. We developed a procedure for the preparation of flavin from liver that was shorter and less laborious than previously used methods, and we also obtained crystals of the flavin. In experiments with both rats and

chicks, we found that our flavin preparation did not prevent the development of dermatitis on a B_2-deficient diet, but another as yet unidentified substance did. Both this unknown substance and the flavin we had isolated were necessary for growth (33).

After a year with Philip Shaffer, my fellowship was extended a year by the International Health Division of the Rockefeller Foundation, and in 1936 I had the opportunity to work in the laboratory of David Keilin, Institute of Parasitology, University of Cambridge, England. Professor Keilin was not a parasitologist, but had his laboratories in the Institute of Parasitology. He was at that time a leading investigator in the study of the cytochrome enzymes that play a major role in tissue respiration. I shared a laboratory with another American, Carl Baumann, who had also received his graduate training in the Department of Agricultural Chemistry at Wisconsin. Professor Keilin assigned us to work on the role of fumaric acid in tissue respiration; again, this included extensive use of Warburg manometers.

At Cambridge we studied liver and kidney tissues of rabbits and heart muscle from pigeons and pigs in order to test the fumarate-oxaloacetate catalytic hypothesis of tissue respiration. We found that the oxygen uptake of these tissues was increased by the addition of very small amounts of fumarate and that this increase was much more than could be accounted for by the oxidation of the fumarate itself. Substances that yielded fumarate on contact with tissues, such as succinate, malate, oxaloacetate, had a similar action (35).

During the Christmas holiday of 1936, my late wife and I spent about 10 days in Germany living with a German family, primarily so we could improve our knowledge of German. Also, because I had used Warburg manometers so often in studies of tissue respiration, I wanted to meet the man who had devised them, Otto Warburg of Berlin.

Our studies on fumarate and succinate were forerunners of the now well-known Krebs citric acid cycle. Indeed Baumann and I often discussed hypotheses of tissue respiration with Hans Krebs, because during much of 1936 he worked at the Department of Biochemistry at Cambridge, just across the courtyard from the Institute of Parasitology. Another frequent visitor to Cambridge that year was Albert Szent-Gyorgyi of Szeged, Hungary. One day after I had given a seminar, Szent-Gyorgyi asked if I would like to spend some time at his laboratory in Szeged. I replied that I would, but I doubted my fellowship would be extended for a third year. He said he thought he could arrange that, and he did.

This third postdoctoral year in Europe was divided half in Szeged and half with Paul Karrer at the University of Zurich so that I might learn some organic chemistry. In Szeged, I continued manometric and chemical experiments on the roles of succinate, fumarate, and oxaloacetate in the respiration of liver

and kidney tissues of rabbits (34). At the end of three years of study in Europe, I returned to the University of Wisconsin as a research associate in biochemistry at the Bowman Cancer Research Institute, later to be renamed the McArdle Cancer Institute. Here, I continued research on tissue respiration with Carl Baumann who had also returned to Wisconsin (35).

MEDICAL SCHOOL

After two years of additional work at the University of Wisconsin, I left to enter the University of Chicago School of Medicine as a third year student in the fall of 1939. Except for anatomy, the course work I had taken as a graduate student met the requirements for the first two years of medicine. I made up the requirement for anatomy by special instruction during the summer of 1938. As a medical student, I was able to find enough time to continue some tissue respiration studies (38) in the laboratory of Alberto Guzman Barron, who worked at the Albert Lasker Research Laboratories at the University of Chicago School of Medicine. These studies led to a review with Dr. Barron in the *Annual Review of Biochemistry* (1).

Perhaps of more importance for my future, by working in the Lasker Laboratories, I had the opportunity to meet Albert Lasker and his charming wife, Mary. Mary Lasker and I became good friends, particularly when we discovered that we both came from nearby small towns in Wisconsin, she from Watertown and I from Columbus. For years our two high schools had been rivals in basketball.

After receiving my MD from the University of Chicago in 1941, I spent a year as an intern in general medicine at Barnes Hospital in St. Louis. That year, I could find no spare time for any research, even though Philip Shaffer's laboratories where I had worked a few years before were just across the street. Near the end of my internship I took and passed the Missouri State Medical Examinations. If I had difficulty getting a job, I could always move to the Ozarks and practice medicine to support my family!

APPOINTMENT AT HARVARD

In February of 1942, after three visits to the Harvard Medical School during which I presented two seminars in the Department of Biochemistry, whose chairman was A. Baird Hastings, I was told that I would be considered for an appointment at Harvard.

At that time, there was no department named "Nutrition" in any health or medical center in the world. Most of the pioneering research in nutrition had been carried out in schools of agriculture, medicine, public health, and in universities by men and women who were in departments of chemistry,

physiology, pathology, and bacteriology. In 1940, C. K. Drinker, dean of the Harvard School of Public Health, and A. Baird Hastings prevailed upon the Rockefeller Foundation to provide funds to establish a Department of Nutrition to be jointly in the schools of medicine and public health. It was specified that the chairman of this new department should have some training in medicine as well as in nutrition and biochemistry, should have had some experience in foreign laboratories, and also have an interest in public health. The department was to organize and supervise the teaching of nutrition in both schools. To my delight, I was invited by Harvard to take on that task and on July 1, 1942, became an assistant professor of nutrition and chairman of this new department. I selected two young scientists, D. Mark Hegsted and John M. McKibbin, as my first academic appointments in the Department of Nutrition. Both had received PhD degrees from the University of Wisconsin in nutritional biochemistry.

In 1942, our country was at war and much of the research in universities related to problems associated with the war. Our initial war-related research dealt with malaria and with the improvement of total parenteral nutrition. Because malaria was a disease of worldwide importance to public health, and was prevalent in many areas where the war was underway, it was important to determine whether atabrine, a relatively new and commonly used antimalarial drug at that time, in any way affected nutritional status and whether nutritional status, particularly certain vitamin deficiencies, affected the efficacy of atabrine. From 1942 to 1946, using ducks and chicks, we did several studies in this area but all essentially with negative results (10). The only convenient experimental animal susceptible to malaria is the duck. The chick, on the other hand, is relatively resistant to malaria. Thus, we compared the response to the same malarial parasite *(Plasmodium lophurae)* of a resistant species and a susceptible species fed identical diets including diets deficient in nicotinic acid, thiamin, choline, and vitamin A. In ducks or chicks, none of these deficiencies influenced the course of the malaria to a significant degree (31).

Our other war-related effort was the improvement of total parenteral nutrition (TPN). I became interested in this problem when I interned at Barnes Hospital in St. Louis. When I arrived in Boston in 1942, the Brigham Hospital had a shortage of physicians. The hospital had just appointed George Thorn as chief of the Department of Medicine, and he asked me to attend on the wards until the war was over and some of the Brigham physicians returned.

Another important development that was to influence my career was the formation of the Nutrition Foundation by a number of executives in the food industry and Karl Compton, president of M.I.T. in 1941. They persuaded Glen King, a co-discoverer of vitamin C, who was then professor of chemistry at the University of Pittsburgh, to accept the position of scientific director

and president of the Nutrition Foundation. The goals of this new foundation, with principal support from the food industry, were to conduct an extensive program in education and research in experimental and clinical nutrition. As part of the education program for scientists, the Nutrition Foundation created a new journal, *Nutrition Reviews,* to provide an authoritative and unbiased review of worldwide progress in the science of nutrition. Dr. King invited me to be the editor of this new journal. I was pleased to accept, and the first issue was published in November 1942. My first editorial board consisted of ten working scientists in the field of nutrition and included Elmer Stotz at Harvard, Carl Moore at Washington University, St. Louis, and Max Wintrobe at the University of Utah, Salt Lake City.

At the time, the major difficulty in total parenteral nutrition was an inability to provide adequate amounts of calories. We tried to increase the calories available to persons on TPN by developing emulsions of fat that could be given intravenously.

RESEARCH DURING THE WAR

From 1942 to 1946 research on parenteral nutrition was generously supported by the US Army and for the next several years by the Upjohn Company. Our very active program to improve fat emulsions for TPN was initially directed by John McKibbin, who left us in 1945 to accept a faculty position at the University of Alabama School of Medicine where he later became professor and chairman of the Department of Biochemistry. I promptly recruited Robert P. Geyer, who had recently obtained his PhD in nutritional biochemistry from the University of Wisconsin, to carry on this research.

Our first published report on fat emulsions for intravenous use appeared in 1945 (19). In dogs, refined coconut oil emulsions stabilized with purified soybean phosphatides were the most successful in our infusion studies. Over the course of the next several years a total of 38 research and review papers in this area came from our laboratories. Our last paper on this subject, published in 1960, was by Dr. Geyer (6). In it, Dr. Geyer concluded, "Although oral feeding is still to be preferred wherever possible, parenteral nutrition has achieved a permanent place in both therapeutic and experimental applications."

From 1942 to 1945, our war-related research was generously supported by various branches of our government. With these funds our laboratories were greatly expanded, not only physically but also in staff. The physical expansion was made possible because Harvard University had bought the Huntington Hospital at 695 Huntington Avenue, near the medical school and school of public health, in Boston, and the Department of Nutrition moved into this new space. But with the reduction of funds for war-related research in 1946 and

with an expanded department, it was necessary either to drastically reduce our activities and staff or find other sources of research funds.

RAISING FUNDS FOR NUTRITION RESEARCH

I opted to raise additional funds and decided to concentrate on various aspects of atherosclerosis and cardiovascular disease. There were several reasons for this decision: Atherosclerosis is an important part of the etiology of coronary heart disease, hypertension, and cerebral hemorrhage, the principal causes of death in our country; obesity is also known to be a hazard in these conditions, as well as in many other illnesses. Since nutrition played a role in these diseases, I thought there might be a good chance of attracting private support from the food industry. Financial support for nutrition research from the latter was already being obtained through the Nutrition Foundation.

As Editor of *Nutrition Reviews,* I was invited to the twice yearly meetings of their Board of Trustees and the annual meeting of their Food Industry Advisory Committee. The former consisted of the presidents of several of our largest food companies and the latter of their directors of research. This obviously provided me with opportunities to seek funds at the highest level in those companies that formed the Nutrition Foundation. In seeking support from the food industry, I had to emphasize that we were not a department of food technology but of nutrition and that any support they might provide had to be unrestricted for the support of both our teaching and research. Eventually, I succeeded in convincing some of the leaders of the food industry that they should consider it an obligation as well as an opportunity to support research in nutrition not only at Harvard but at other academic nutrition centers.

As mentioned earlier, when I was a medical student at the University of Chicago, I spent some time in the Lasker Laboratories and had met Mrs. Albert Lasker. After moving to Boston in 1942, occasionally I would see Mrs. Lasker when I was in New York. When support for war-related research began to lessen, it was natural for me to ask the Lasker Foundation for help. Beginning in the late 1940s, and for many years thereafter, the Lasker Foundation was very generous in providing unrestricted funds for our research.

TISSUE RESPIRATION AND VITAMINS

After the war I decided to extend my earlier work on "respiration and vitamins." Now that we were receiving generous, unrestricted private support (this was before the days of NIH grants), it was possible to continue some activities in tissue respiration; but because the long-range goal was

"heart disease," I wanted to conduct research on basic factors affecting the respiration of heart muscle. In the mid 1940s, I recruited a bright, young postdoctoral fellow, Robert E. Olson, who was interested in metabolism and nutrition. I encouraged him to undertake studies of respiration of heart tissue available from ducks and chicks that had been made deficient in various vitamins. Olson et al found that the pyruvate utilization by cardiac muscle from rats and ducks is depressed in thiamin deficiency and that the addition of thiamin in vitro tends to restore pyruvate utilization to normal (26). Dr. Olson continued these respiration studies with heart muscle tissue from rats and ducks deficient in biotin, pantothenic acid, and pyridoxine (2, 22–28). Dr. Olson went on to chair departments of biochemistry and nutrition at the University of Pittsburgh and St. Louis University and eventually became the editor of *Nutrition Reviews*.

More on vitamins! By the mid 1940s, Dr. Hegsted had formed a friendly and cooperative arrangement with S. B. Wolbach, who was then professor and chairman, department of pathology at Harvard Medical School. In 1952, they reported that the skeletal responses to hypervitaminosis A in young chicks are precisely of the same nature as those in mammals (44). As in mammals, hypervitaminosis A in growing chicks accelerates all histologic sequences in bone growth in conformity with the normal growth patterns.

Several papers dealing with vitamins A, D, E, ascorbic acid, folic acid, and vitamin B_{12} were published by members of our department; the last one was published in 1985 (5).

CHOLESTEROL AND ATHEROSCLEROSIS

The first paper on cholesterol and atherosclerosis from our department was published in January 1953 by George V. Mann (17), a research associate. In the first sentence of that paper the author noted, "The factors that control cholesterol metabolism are poorly understood." Dr. Mann had also been involved with some of our studies with fat emulsions given intravenously and he wrote: "Chance observations of the effects of infusions of fat emulsions in dogs suggested that the cholesterol content of the blood was sensitive to stress [generated by intravenous fat emulsions] and that the esterified cholesterol component was particularly influenced by such stress."

Dr. Mann was also an author of our second paper in this area, published in June 1953 (41). To quote the first and last sentences of that paper published almost 40 years ago: "Determination of the role of weight reduction as a preventive and therapeutic measure in the control of atherosclerotic vascular disease is of considerable practical importance. . . . If elevated serum lipid levels contribute to the causation of atherosclerosis, weight reduction is a proper treatment for this disease."

Productive study of atherosclerosis or any disease is greatly facilitated if an animal model can be found that resembles the human and can be managed under laboratory conditions. For atherosclerosis such a species should have a susceptibility to the development of arterial disease resembling that seen in humans. The necessary and crucial transfer of information to humans would also be facilitated if the dietary habits of the experimental species resembled those of man. Certain species of subhuman primates would appear to be a likely choice, yet it was widely believed in the late 1940s that atherosclerosis is rarely seen in subhuman primates as a naturally occurring disease, and further that these animals are resistant to the experimental induction of atherosclerosis. Our initial studies of atherosclerosis in monkeys were done primarily by George V. Mann and by our pathologist, Steven B. Andrus.

We had intended to use rhesus monkeys, but soon found that they were nearly impossible to obtain and very expensive, because in the late 1940s the pharmaceutical companies were purchasing most available rhesus monkeys for use in making Salk polio vaccine. Hence, we decided to use the ordinary "organ grinder" monkey obtainable from a variety of pet shops. They were imported from Latin America, mostly from Colombia, and were Cebus monkeys *(Cebus albifrons)*. The results of our first studies using monkeys were published in 1952 and 1953 (14, 16). To our knowledge, this was the first report of the experimental production of atherosclerosis by dietary measures in a subhuman primate.

We had produced the disease by feeding a purified diet high in cholesterol and low in sulfur amino acids over periods of 18 to 30 weeks. Within 2 to 8 weeks, this regimen caused the total serum cholesterol to rise to 300 to 800 mg/dl from the usual value of 140 mg/dl. The hypercholesterolemia could be largely prevented by feeding 1 g per day of *dl*-methionine or *l*-cystine as supplements to the diet. The vascular lesions were in the ascending aorta but extended from the valves of the left ventrical to the proximal portions of the carotid and femoral arteries. Minimal lesions were observed in the coronary arteries. Visceral cholesterolosis was not associated with this disease.

Having found that the hypercholesteremia we produced in monkeys could be largely prevented by adding supplements of methionine or cystine to the diet, we hastened to conduct human studies, but our results were negative (15). In 24 American males with moderate to marked elevations of serum cholesterol, 3 g of methionine added daily to their usual diet at mealtime for six weeks did not alter their serum cholesterol and lipoprotein levels.

Cooperative Study on Lipoproteins and Atherosclerosis

A major paper on cholesterol and beta-lipoproteins in the serum of healthy Americans and of those with coronary heart disease was published by members of our department in 1957 (11). During the period May 1951 through June 1954, serum lipids of 2,045 human subjects were examined as part

of the Cooperative Study of Lipoproteins and Atherosclerosis (20). Our part of this study was concerned with 1,968 adults of various ages who were active and healthy as compared with 273 men who had experienced a myocardial infarction, 141 men with angina pectoris but without myocardial infarction, and 23 women with myocardial infarction—a sizable sample for a nonepidemiological study.

Our measurements of various lipoproteins and total cholesterol were related to sex, age, and body weight. We reported that the most characteristic attribute of serum lipid measurements in adults of similar age, sex, and clinical status is their large variability. Among healthy people under 50 years of age, men show higher levels of all these serum lipids than do age-matched women. The age trend of serum lipid levels is different for the two sexes. Women show a steady increase in levels with age throughout the age span studied. After age sixty, serum cholesterol levels of women frequently exceed those of men. The trends of serum lipid levels with age are partly attributable to fattening with age.

The 273 men with established myocardial infarction were found to have both serum cholesterol and lipoprotein levels that were higher on the average than those found in age-matched men without obvious disease. This finding supports the belief that clinical manifestations of atherosclerosis are associated with a disorder of lipid metabolism. The serum lipid levels of the 23 women with myocardial infarction were similar to those of men with the same disease and were higher than age-matched women without obvious disease. The serum lipid levels of the 141 men with angina pectoris only were intermediate between those of healthy men and men with myocardial infarction. The small size and great variability of these differences of serum lipid levels between well men and women and those with angina pectoris or myocardial infarction prevent efficient application of serum cholesterol and lipoprotein levels *by themselves* to the clinical prediction of coronary heart disease among individuals.

Clinical Studies

In the early 1960s, we made arrangements with the Danvers State Hospital, a mental institution located about 25 miles north of Boston, for a long-term study on the quantitative effects of dietary fat on serum cholesterol. Dr. Hegsted and others of our staff implemented this study. We provided a separate kitchen and dining room for our subjects who were all males. Selected from a large group, most of our subjects were chronic schizophrenic patients without evidence of physical disease and were between 34 to 57 years of age. Those with serum cholesterol values above 300 or lower than 200 mg/dl were excluded. The final selection was based primarily upon the

probability that they would be available and cooperative over a long period of time. All the men were housed and fed in an isolated ward that included a recreation room in addition to the kitchen and dining facilities. They continued their usual supervised activities. The purpose of the study and the need for adherence to the diet were continually stressed to attendants and subjects. A dietitian from our staff supervised preparation of all meals.

The men were studied in groups of ten. They were fed a low-fat diet to which various fats were added. The data were analyzed to determine the serum cholesterol response to various fatty acids. An unusually strong feature of these studies was that the comparisons were made with the same men throughout the study. The test oils were used primarily by incorporating them into recipes for many products such as waffles, muffins, cakes, pie crust, biscuits, salad dressings, and spreads for bread. Considerable experimental work was necessary to obtain satisfactory products with certain oils. Filled milk and ice creams prepared from nonfat milk and the appropriate oil were used. Including the control diet, a total of 36 different fats and oils were used, each for a period of four weeks and each experimental diet was preceded by a four-week period on the control diet. The range of total fat in the diets varied from low, at 22% of total calories, to high, at 38 to 40%.

Among the more interesting results (9) are the following:

1. Approximately 67% of the total variance in the level of serum cholesterol was explained by changes in the dietary content of myristic acid alone. This appears to be the most important of the fatty acid components affecting total serum cholesterol levels.
2. Palmitic acid has significant but much lesser effects upon the level of serum cholesterol than does myristic acid.
3. No specific effects on serum cholesterol could be detected for stearic, lauric, or shorter chain saturated acids or for monounsaturated acids except that their presence in fats lowers the proportions of myristic, palmitic, and polyunsaturated fatty acids.
4. The amount of dietary fat tested between 22 and 40% of the total calories appeared to be without influence upon the level of serum cholesterol.
5. Dietary cholesterol appeared to be linearly related to the serum cholesterol. An increase in 100 mg in dietary cholesterol provoked a rise of serum cholesterol of approximately 5 mg/dl. This response was independent of the effects induced by dietary fat.
6. In view of these results we proposed that the most effective practical diets for lowering serum cholesterol should be those relatively high in total fat with a small proportion of myristic and palmitic acids, particularly myristic acid, a high proportion of polyunsaturated acids, and a small amount of cholesterol.

Experimental Studies

In the 1960s our experimental studies continued in parallel with these clinical studies. While our initial studies of diet and atherosclerosis in nonhuman primates were with *Cebus albifrons*, we soon began to use other species of New World monkeys. In fact, we sponsored an expedition to the jungles of Colombia to see how many different species of monkeys could be obtained, and then studied at autopsy 73 vascular specimens from Cebus and 4 other species of New World monkeys. We found substantial differences in the frequency and extent of early arterial lesions. When different species were studied in the laboratory, we found different responses to arterial lesions and levels of serum cholesterol with the same diets (30).

One of the puzzling aspects of atherosclerosis has been the occasional discrepancy between hypercholesterolemia and the incidence of atherosclerosis or ischemic heart disease. Whereas hypercholesterolemia is considered a primary risk factor in atherosclerosis, some individuals with elevated levels of cholesterol do not develop significant atherosclerotic vascular disease while other normocholesterolemic persons do.

In 1977 we reported that squirrel and Cebus monkeys fed a coconut oil diet develop comparable hypercholesterolemias, but the squirrel monkey primarily increases its high-density lipoprotein (HDL) pool of cholesterol (21). These results, coupled with the greater accumulation of aortic lipid, particularly cholesteryl ester, in the atherosclerotic-susceptible squirrel monkey, support the concept of the protective nature of high-density lipoproteins and the atherogenic potential of low-density lipoprotein (LDL). They also suggest that a species' genetic control of the lipoprotein response to diet is variable, which has important biological implications.

Our use of several species of New World monkeys greatly expanded, not only for research related to atherosclerosis but also for research on other problems concerning nutrition. Therefore we started breeding our own monkeys, first in Colombia, then in Guatemala, and finally in our own animal facilities in Boston. Here, for several years, an average of about 100 baby monkeys were born each year. Having a good supply of infant monkeys enabled us to study a number of psychological and behavioral problems during growth and development. Stephan & Hayes published one of our last papers related to diet and cholesterol in monkeys in 1985 (40).

Boston-Ireland Brothers Study

Any review of the contributions of our laboratory to studies of diet, atherosclerosis, and coronary heart disease should mention our Boston-Ireland Brothers Study. This was a large cooperative study with Trinity College School of Medicine, Dublin, Ireland, carried out in the 1960s and published

in 1970 (3). It was a nutritional and epidemiological study involving 1,994 middle-aged men including over 500 pairs of brothers, one of whom still lived in Ireland whereas the other had lived in Boston for at least 10 years. All brothers were within 10 years of age of each other. The intake of calories, complex carbohydrates, magnesium, and fluoride (from tea) was higher in Ireland. The proportion of calories derived from fat and saturated fat, the serum cholesterol, blood pressure levels, and the amount of cigarette smoking did not differ markedly. The weight, skinfold thickness, and number of abnormal electrocardiograms were higher in the Boston subjects. A study of the pathology of coronary arteries and aortas from autopsies (of other individuals) revealed much earlier serious atheromatous involvement in Boston Irish than in Irish specimens. Increased physical activity appeared to be most important in reducing the risk of coronary heart disease in Ireland.

Although the major interest in atherosclerotic vascular disease has been directed toward males in the fifth decade of life and older, the period in which clinical complications become increasingly common, a consideration of the prolonged course of the underlying atherosclerosis suggests that attention aimed at prevention must ultimately be turned to the second decade. We were among the first to study the role of diet on total serum cholesterol levels in adolescent boys. Our aim was to demonstrate whether practical, realistic, acceptable modification in the usual diets of adolescent males could effectively prevent or lessen the usual rise in plasma cholesterol levels that characterizes American adolescent males. We began with no preset criteria or dietary goals, but instead aimed at making changes within limits of acceptability. Furthermore, we were not working in a metabolic ward situation and therefore interpreted the outcome against a background of considerable and variable nonadherence.

Study of Adolescent Boys

In the fall of 1969, we obtained cooperation of the administration, faculty, students, and parents of a boys' boarding school located an hour's drive from Boston. Much of this cooperation was due to the assistance of an internationally recognized cardiologist and Harvard professor, Dr. Paul Dudley White. The school had a student body of approximately 220 boys aged 13 to 18 years. The school was located about 3 miles from the nearest source of off-campus food, thus at least minimizing a major source of nonadherence. Meals were prepared in the school kitchen under our supervision. The first few months were spent in trying out various diet modifications at a "test table," where only nine boys ate, and gaining the cooperation of a few food companies to prepare special foods. In January 1970, before introduction of diet modifications to the entire school population, serum cholesterol determinations were made. They were repeated before and after the Easter

vacation in the spring of 1970, again when the boys left for summer vacation, and also when they returned in the fall of 1970. We showed clearly that blood cholesterol, a risk factor for atherosclerosis, is readily lowered by realistic and acceptable modifications of dietary fats and cholesterol. Among boys whose baseline levels of total cholesterol were 200 mg/100 ml or higher, the average reduction was 15%; in those whose baseline level was 199 mg or lower, the mean decrease was 8%. As far as we know, this study represents the first attempt at lessening one of the risk factors associated with atherosclerotic vascular disease in later life at the time of adolescence when it may begin to develop (18). This study was repeated in 1970–1971 in a larger boarding school (448 students) with similar results (4).

From these two studies, it appears that the most significant dietary changes to initiate in feeding adolescent boys in order to lower serum cholesterol levels are, in order of importance:

1. Use of a low-fat milk with extra skim milk solids
2. Replacement of butter with a highly polyunsaturated margarine
3. Use of polyunsaturated oils and shortenings in baked goods and for frying
4. Use of low cholesterol or cholesterol-free egg products whenever possible, particularly for baking
5. Fewer eggs and more cereals
6. Use of a low-fat ice cream or an ice cream made with a polyunsaturated fat
7. Use of fish, veal, poultry, and carefully trimmed beef with some frequency

These suggestions should be given serious consideration by institutions, whether private or public, when planning menus for feeding adolescent boys and for any modification of existing legislation for school-feeding programs.

Abundant evidence is available that the risk of developing coronary heart disease is positively correlated with the plasma cholesterol level in men from 30–35 up to 60–65 years of age when the total cholesterol is 240–250 mg/dl or higher *and other coronary risk factors are present*. Evidence for similar findings beyond 65 years of age is skimpy.

The efficacy of diets altered in total calories, fat, and cholesterol in lowering plasma cholesterol has been demonstrated for more than three decades. The American Heart Association and the National Heart, Lung, and Blood Institute have urged Americans to modify their dietary habits. A logical application of these suggestions infers a change in family food choices. The cholesterol response of family groups to altered dietary regimens has seldom been studied. We were one of the first groups to report a study in which families participated in a dietary study for the purpose of lowering total serum cholesterol (43).

Boston Family Study

We recruited 46 families in a short-term (7 week) study. A family was defined as including an adult man, an adult woman, and one or more adolescents, all living at home and eating most of their meals there. The study consisted of two parts: a four-week baseline period and a three-week diet-change period. Each family was assigned a nutritionist who assisted with dietary problems and maintained contact throughout the study.

In this short-term, professionally supervised study, in which dietary cholesterol and saturated fat sources were decreased and sunflower oil and margarine were added as the major sources of polyunsaturated fat, an average reduction in serum cholesterol approximating 10% was achieved within 10 days and maintained throughout a three-week period. Adult and adolescent females had slightly greater cholesterol-lowering responses than did adolescent and adult males.

The high degree of cooperation among the free-living families in this study has positive implications for enlisting complete family involvement in preventive and therapeutic dietary programs. It should be remembered, however, that this was a short-term study and it was carefully and frequently monitored by a trained nutritionist.

A final blood sample was obtained on all but one of the study participants approximately 14 weeks after the test-diet ended. Serum cholesterol had returned to, or surpassed, baseline values. These results indicate the rapidity with which achieved goals may be negated and the need for continued adherence and monitoring if reductions in serum cholesterol are to be maintained.

NUTRITION EDUCATION

So far, I have commented briefly on a few of the research papers dealing with "respiration, vitamins, cholesterol, and atherosclerosis" from Harvard's Department of Nutrition, but the department has also been active in a variety of nutrition education pursuits. I would like to mention a few of them.

From the early 1940s, various members of the department prepared the sections on vitamins and nutrition in several encyclopedias, including the *Encyclopedia Britannica* and the *Britannica Book of the Year*. In 1952, at the request of the American Heart Association, we prepared a booklet, "Food for Your Heart, A Manual for Patient and Physician" (39). It was reviewed and endorsed by the Council on Foods and Nutrition of the American Medical Association. It has long been out of print and as of a few years ago I was interested to learn that neither the offices of the American Heart Association nor the National Heart, Lung, Blood Institute knew of its existence. It might

be of interest to quote a few sentences: "Nutrition and food have a two-way relationship to normal heart function and to high blood pressure. First is the prevention or treatment of overweight or obesity—the result of eating more food energy (calories) than the body needs. Second is the treatment of hypertension. . . . There is little point in comparing the nutritive value of single foods because most foods supplement the nutritive value of others. The fundamental rules of good nutrition are these: First, eat a variety of foods; second, maintain your desirable weight."

The concept of the Basic Four Food Groups came from our department and was presented at the 38th Annual Meeting of the American Dietetic Association in St. Louis in October, 1955 (8). Two years later, this concept was adopted by the US Department of Agriculture.

Our most recent "education" paper (42) was directed to physicians and questions the efficacy and cost efficiency of the National Cholesterol Education Program, which recommends that the entire US population reduce dietary intake of saturated fats and cholesterol to reduce the risk of coronary heart disease (CHD). The role of diet in the causation of CHD remains unresolved, and overemphasis on diet may shift attention away from more promising regimens, such as quitting smoking, controlling hypertension, diabetes, and stress, and maintaining a reasonable body weight and a moderate level of physical activity.

THE LOWN RESEARCH GROUP

No comments about the activities of Harvard's Department of Nutrition related to heart disease would be complete without mentioning the outstanding activities of the "Lown Group." They have not been discussed thus far in this prefatory chapter because they do not deal primarily with nutritional aspects of atherosclerosis. Bernard Lown joined our group in 1958 as a research associate in medicine, in part to give us a more balanced approach to interpretation of our animal work relative to cholesterol and atherosclerosis and its application to heart disease in man. He and his group helped us gain the general acceptance of our department by the medical profession. Some years ago, Dr. Lown was promoted to professor of cardiology in public health. He gathered around him a group of brilliant young postdoctoral fellows whose productivity over the years has been tremendous, approximately 400 research and review papers. I shall mention only two of his papers. The first deals with the development of a method for terminating cardiac arrhythmias (13) that utilizes an electronic device employing direct current discharge. Transthoracic DC countershock was found to be 100% effective in controlling 550 episodes of ventricular fibrillation in 20 dogs, all of which survived. It was then used to treat 25 episodes of arrhythmias in 19 patients, and 23 of the 25 episodes were successfully reverted. The Lown Cardioverter

is now standard equipment in most hospitals and in many ambulances. It is truly a life-saving device.

The second paper from the "Lown Group" that I mention is very simple, yet the recommendations made in it are commonly accepted today as part of the treatment of myocardial infarction (32). Myocardial infarction and its complications evoke the highest toll of human life of any single disease in the United States. Over the years, many different forms of therapy have been introduced. A vast array of drugs and sophisticated electronic devices have been developed, and these have important uses. However, one of the simplest, least expensive, and possibly most effective modes of therapy is the so-called "arm chair" treatment of myocardial infarction.

Prior to this simple procedure, complete bed rest had been the rule in treating patients with acute myocardial infarction, despite the lack of convincing evidence that this is beneficial to the heart. The "arm chair" treatment is based on the belief that strictly enforced bed rest is more taxing to the patient and to the damaged heart than a sitting position in a comfortable chair.

This report dealt with 184 patients with acute myocardial infarction who were admitted to a coronary care unit over a two and one-half year period and who were helped into an arm chair, most within 1–2 days of admission, all within one week, and daily thereafter. Patients tolerated this treatment well. Clinically, important changes in blood pressure and pulse were seldom observed. These results show that arm chair treatment can be utilized in patients with acute myocardial infarction without adverse effects upon the circulation and with great benefits to the patient's morale.

An accomplishment of Dr. Lown that has nothing to do with "respiration, vitamins, cholesterol, or atherosclerosis," but that, in part, involved our department and may be more important than all research from this department, was his founding of the International Physicians for the Prevention of Nuclear War (IPPNW) in 1979 in cooperation with his cardiology colleague in Moscow, Evugene Chazov. Drs. Lown and Chazov had collaborated in cardiologic work for more than 20 years. Now, they collaborated in developing a society for the prevention of nuclear war. IPPNW was formally organized in the seminar room of Harvard's Department of Nutrition in 1979. It now has groups in many cities and in 70 countries and concerns itself with only one issue: prevention of nuclear war. In 1985 IPPNW was awarded the Nobel Peace Prize, and it was accepted by Drs. Lown and Chazov (12).

CONCLUSION

My comments have been mainly confined to "respiration, vitamins, cholesterol, and atherosclerosis" and are autobiographical in part as requested in the invitation to prepare this prefatory chapter. I have confined this chapter to a review of a few of my papers published as a graduate and postgraduate student

on these subjects and also to a few of the papers on these four subjects from scientists who were members of Harvard's Department of Nutrition from 1942 to 1986. In 1987, I prepared a monograph titled, "Harvard's Department of Nutrition 1942–86." It lists the complete references of all papers published from this department during that 44-year period, a total of 2,250, divided into various categories, and is preceded by a brief history of the department, followed by a listing of all sources of support divided into private, government, and industry.

Professor A. Baird Hastings was probably the key figure in motivating the International Health Division of the Rockefeller Foundation to provide a five-year grant that made possible the founding of Harvard's Department of Nutrition. He also helped nurture the department for its first few years. In his autobiography published in 1989 he states, "It is still my conviction that the training of able scientists is the way to secure progress in science, and I must confess that I am personally much prouder of the M.D.'s and Ph.D.'s whose scientific education and research activities I have had the privilege of helping than I am of the papers I have published myself" (7).

This chapter closes in the same spirit. I am more proud of the men and women who as students, postdoctoral fellows, staff members, and technicians were trained in Harvard's Department of Nutrition, in part by our senior staff but largely by themselves, in facilities and with fellowships and salaries provided by the department from funds raised largely by me, than I am of any studies suggested or done primarily by me. And they were trained in many aspects of nutrition other than respiration, vitamins, cholesterol, and atherosclerosis.

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