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Cholesterol-Watching: [Dr. Edward H. Ahrens, Jr.]

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THE ROCKEFELLER UNIVERSITY

RESEARCH

PROFILES

I think of a clinical investigator as one trying to ride two horses – attempting to be an investigator and a clinician at one and the same time. Whereas such an equestrian manoeuvre is usually considered a bad policy, in this case...experience has shown that it is a very fruitful pastime.

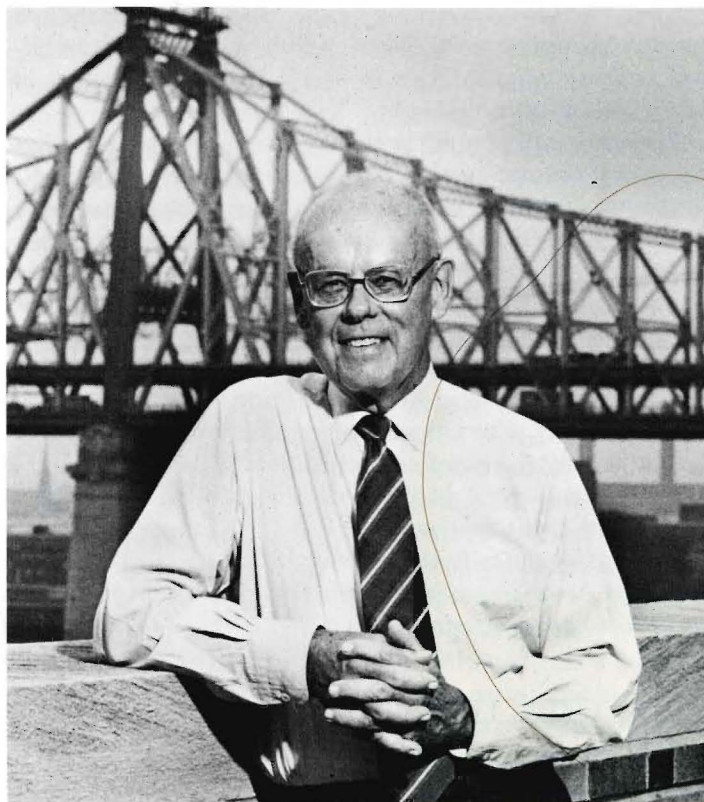
FULLER ALBRIGHT, 1984

FALL 1984

Cholesterol-Watching

The fatty alcohol cholesterol, wrapped in molecules of lipoproteins, travels through the bloodstream to the body's cells to repair membranes and make steroid hormones. Other lipoproteins – proteins with fats, or lipids, attached to them – remove cholesterol from tissues and take it back to the liver where it is processed for disposal. When cholesterol metabolism is functioning normally, the liver makes most of the cholesterol the body uses. The cholesterol absorbed from food restrains the liver's production of cholesterol, keeping the supply in balance with the body's needs. But as no one today needs reminding, sometimes cholesterol metabolism doesn't function normally. In the process of atherosclerosis, for example, cholesterol builds up on the walls of arteries and blocks the life-sustaining flow of blood to the heart and other organs.

In the 1940s, when Edward H. Ahrens, Jr. was drawn unexpectedly into the "murky business of lipids," there was circumstantial evidence that dietary fats play a role in atherosclerosis and heart disease but almost no means for investigating this scientifically. In a few years, the picture had changed dramatically. New technologies, developed or refined by Dr. Ahrens and others, made it possible to sort out the confusion of fatty compounds in the body, and in the 1950s his laboratory provided definitive confirmation that the kind of fats we eat can alter the level of cholesterol in our blood. The terms "saturated" and "unsaturated" entered the popular vocabulary and,



Edward H. Ahrens, Jr.



Dr. Ahrens and patient in the Rockefeller University Hospital.

as he later wrote, "the sleepy old field of lipid research took off for the moon." Today he is a spirited participant in the ongoing debate over dietary and other strategies for coping with cholesterol problems.

Dr. Ahrens is Frederick Henry Leonhardt Professor of The Rockefeller University and a senior physician in the University's research hospital where patients with disorders that science still cannot fully explain, prevent, or cure, like atherosclerosis, participate in experiments and receive free treatment and counsel. Trained as a pediatrician, he first came to Rockefeller to study liver diseases. His interest was endocrinology, and the liver is a major processing center for hormones. But the first patient assigned to him on his arrival was suffering from a disease in which cholesterol metabolism had gone badly awry. His initiation into "cholesterol-watching," as he calls it, produced the first detailed description of the disease, which he named primary biliary cirrhosis, and led him to decide that lipids weren't as "charmless" as he had thought.

The major difficulty at the time was in purifying fats: isolating them from one another and from other body compounds. Since some of the best work in separation technology was being done at Rockefeller, Dr. Ahrens spent two years with Lyman Craig, inventor of a method called countercurrent distribution, which separates mixtures into their component parts by differences in their partitioning between two solvents. Down the hall from Craig's laboratory, Stanford Moore and William Stein, later to receive the Nobel Prize, were purifying amino acids by chromatography, a technique that takes advantage of the fact that various closely related compounds adhere differently to materials they pass through. Dr. Ahrens learned both procedures and was the first scientist in the country to adapt gas-liquid chromatography to fatty compounds, an innovation the American Heart Association credited with helping to "revolutionize the field of lipid chemistry."

"FEED THEM LIKE BABIES"

In 1952 he turned to the problem of atherosclerosis. That the quality of dietary fats rather than simply their quantity might

be a precipitating factor had been suggested by Laurance W. Kinsell, an endocrinologist trained by Fuller Albright at Harvard Medical School, the same teacher who had inspired Dr. Ahrens. "Kinsell had observed that animal fats raised cholesterol levels in the blood while vegetable fats lowered them," says Dr. Ahrens, "but since his experiment had been designed to study hormones, with cholesterol thrown in as an afterthought, not too many people took him seriously. I decided to design an experiment specifically to test this point.

"We had an awful time at first. It was boring as the devil for the patients, who had to eat the same diet every day for weeks. It was hard on the dietitians, and we didn't have precise enough control of the dietary mix. I was telling my troubles to one of our elder pediatric statesmen one day, Dr. Emmett Holt, whose father was a member of the first board of The Rockefeller. Holt just looked at me, disgusted. 'I thought you were a pediatrician,' he said. 'Feed them like babies. Feed them formula.' Well, of course, that was the solution and the birth of the formula diets that are used so widely now in clinical experimentation.

"After we found a basic mixture that could stand up to our manipulations, we tested about forty fats on several hundred patients. We not only confirmed that different fats change cholesterol levels, we found out that the effects depend on the degree of saturation." (Unsaturated fats, like vegetable and fish oils, are liquid at body temperature. Saturated fats, like those in meat and cheese, have much higher melting points.)

A young cardiologist named Jules Hirsch, one of the first of many researchers who have come to Rockefeller over the years to work with "Pete" Ahrens, as he is universally known, was on the project and also worked on refining chromatography methods. Dr. Hirsch explains: "Cholesterol occurs in a free state or attached to fatty acids. Other fats in the blood have the same fatty acids, but in different chemical configurations. With chromatography we were able to start breaking down these mixtures and follow them individually, which no one had done before. We were able to determine how fats are distributed in the body and analyze what was happening in the gastrointestinal tract as they were absorbed."

In planning the experiment, Dr. Ahrens hit on the idea of re-

Cholesterol is a major component of cell membranes and steroid hormones, and that part of it converted to bile acids plays an essential role in fat absorption and digestion.

In normal cholesterol metabolism, the daily rate of synthesis in the liver, the principal site of cholesterol manufacture in the body, is equal to the difference between dietary intake and excretion. The average level of plasma cholesterol in the United States is now about 220 milligrams per 100 milliliters of blood plasma. An elevated level of plasma cholesterol is a major risk factor for atherosclerosis.

Malfunctions of cholesterol metabolism can be attacked by means of dietary intervention, drugs, or surgical procedures. All of these act at various points along the metabolic pathway to: modify cholesterol intake (A); reduce absorption (B); enhance the formation of bile acids (C); increase the outflow of tissue cholesterol (D); reduce synthesis in the liver and other tissues (E); or physically remove cholesterol from the blood, as in LDL pheresis (see page 6).

cruiting overweight people, to whom he promised (and delivered) weight reduction. He has since confined his studies to patients whose concern is clearly arterial disease. Dr. Hirsch, still at Rockefeller, went on to direct one of the country's leading programs for the study of obesity.

DELVING INTO THE TREASURE HOUSE

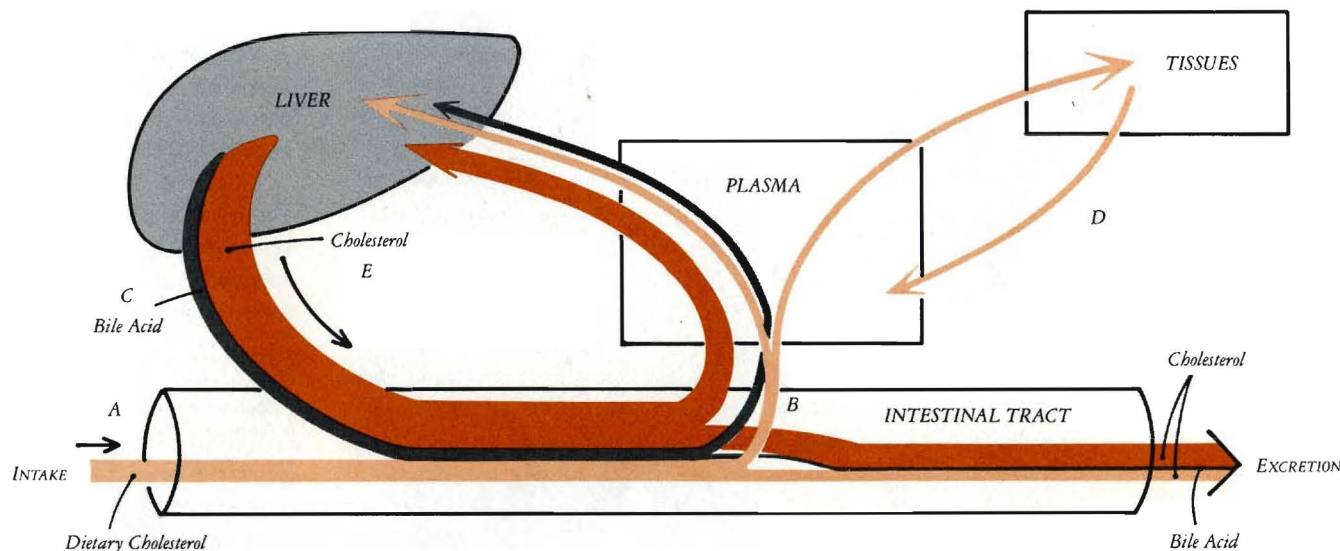
By 1960 Dr. Ahrens was ready to tackle the question of the mechanisms of cholesterol regulation. "We knew we could control the intake of cholesterol with formula feeding," he says, "but to find out how much cholesterol the body was making we had to compare intake against outflow, the route of disposal being the feces. It wasn't an easy job. The feces are a jumble of many materials. Emil Fischer called them the treasure house of biochemistry. Well, we started delving into the treasure house like Midas. We got the gold, but it took a long time.

"To complicate matters, we knew the skin has sterols in it, too, and we didn't know if they would affect our calculations. That worry led us into one of the most trying and bizarre experiments we've ever done. We wrapped a handful of poor souls in specially made suits, head to toe, and every day we'd rub them down, take the clothing to the lab, and measure the fat in it. The worst part for us was preparing the suits. Almost all cloth is made with sizing, which is full of fat. Before we could use the stuff, we had to put it in huge vats of solvent, over and over again for days, to be sure the sizing was out. It was a great relief to learn that cholesterol in the skin is made in the skin and doesn't circulate. We didn't have to do any more laundry."

During this period, researchers elsewhere introduced the practice of feeding patients cholesterol tagged with radioactive isotopes, which makes it easier to track through the body.

After more than a decade and hundreds of experiments on the regulation of cholesterol metabolism, Dr. Ahrens developed a

ROAD MAP OF CHOLESTEROL METABOLISM



technique known as the sterol balance method, which accurately measures the difference between dietary intake and total daily excretion: this difference is due to the body's own manufacture of cholesterol. With sterol balance methods and isotope feeding, he was able for the first time to measure precisely the dietary intake of cholesterol, its absorption from the intestines, conversion to bile acids, body synthesis rates and its feedback control, transfer of stored pools of cholesterol in and out of tissues, and excretion rates. (See diagram, page 3.)

He learned not only *how* cholesterol metabolism is regulated, but also how *differently* it is regulated in different people, a finding he considers his most significant scientific contribution. This concept underlies all the work in his laboratory now and is the basis of the skepticism he has expressed about some of the recent publicity on dietary cholesterol.

"Blood levels of cholesterol are useful indicators of people at risk," he states, "but the important question to ask about such a person, once identified, is *why* the level is elevated; is the body making or absorbing too much cholesterol or not excreting enough? Most treatment is aimed simply at lowering blood lipid levels, which is not necessarily beneficial. Using our sterol balance methods, we saw, for example, that people on an unsaturated-fat diet usually reduce the pool of plasma cholesterol by shifting it from the plasma into the bulk tissues — muscle, connective tissue, and adipose tissue; and with various other manipulations, we showed that the excess cholesterol stored in tissues can be vastly reduced without significantly altering the plasma levels.

"Our aim is to develop further means for pinpointing defects and individualizing treatments in the most appropriate way. Thus, we'd like to see to what extent we can predictably affect the atherosclerotic process or even reverse it if possible, which no one has yet shown can be done."

NEW DIRECTIONS

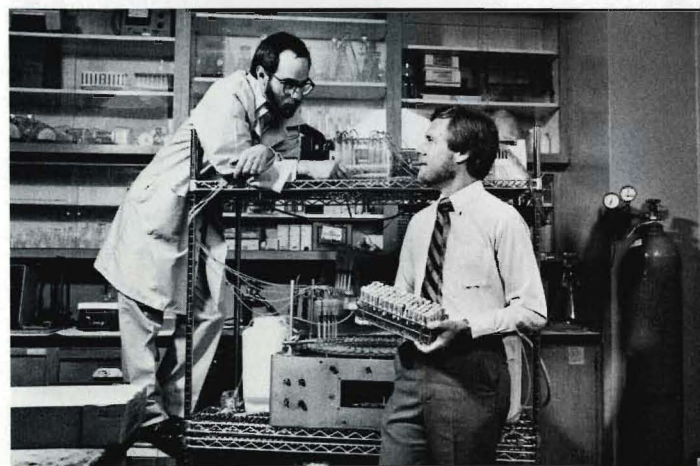
Sterol balance methods require hospitalization for many weeks on a formula diet. Donald J. McNamara, a member of Dr. Ahrens' group since 1974, is a biochemist whose research into the

chemistry and physiology of cholesterol metabolism has led to quicker and easier tests. One of the procedures he has introduced resulted from studies of mevalonic acid that he conducted with Thomas Parker, another biochemist in this lab.

"Mevalonic acid, or mevalonate, is a precursor stage in cholesterol synthesis," Dr. McNamara explains. "Your body must make mevalonate before it can make cholesterol. While we were investigating mevalonate synthesis, Tom and I realized we could put it to work clinically. In the method we've designed, we feed a patient a high-cholesterol diet and take a blood sample. If we see a change in the level of mevalonate compared to baseline levels, we know that a change in the cholesterol level is the result of a change in synthesis. The whole thing takes twenty minutes."

Cholesterol metabolism has rarely been studied in children or in women of child-bearing age because of the potential danger of using radioactive tracers. Lisa Hudgins, the newest member of the laboratory, has synthesized a non-radioactive tagged cholesterol; its effectiveness is currently being checked against results obtained with radioactive isotopes.

"When we're sure it works," she says, "and at this point it looks good, we want to start by measuring cholesterol absorption, which hasn't been measured in normal infants or children. In today's world, parents have been advised to feed their children



Thomas Parker, left, and Donald McNamara with their apparatus for measuring concentrations of mevalonic acid in plasma samples from patients. Mevalonic acid is a precursor stage in the synthesis of cholesterol.

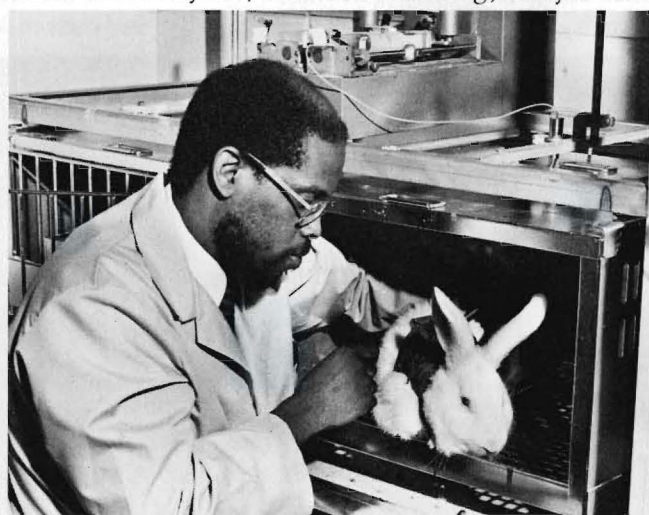
Lisa Hudgins and a patient-volunteer consult on the sun deck of the Hospital. Dr. Hudgins, who is currently studying cholesterol metabolism in children, is concerned with the question of how the process changes with age.

Clinton Brown recently conducted an experiment in which a patient was fed intravenously with a diet containing a substance which may act to draw cholesterol out of tissues. In conjunction with the clinical study, Dr. Brown, shown here in the Laboratory Animal Research Center at Rockefeller, administered the substance to hypercholesterolemic rabbits whose arteries will be dissected and compared with a control group of animals.



a low-cholesterol diet, but in fact we don't know to what extent infants absorb cholesterol, or whether there is a difference in absorption between normal children and children from families with histories of heart disease. We want to learn how cholesterol metabolism changes with age. We want to study a spectrum of families with different health histories. We want to find out if functional differences can be identified early in life."

Dr. Hudgins, a pediatrician like Dr. Ahrens, came to Rockefeller as a clinical fellow, a program established at the hospital in 1976 to provide young physicians with opportunities for hands-on experience in experimental medicine. Such opportunities have been drastically curtailed throughout the country in recent years. It's a subject Dr. Ahrens feels strongly about. In a retrospective article he wrote this past summer for the twenty-fifth anniversary volume of the *Journal of Lipid Research* (a publication he founded and which he edited during its first years), he recalled that the strongest impression of his own initiation into the field was "the realization of how rewarding patient-oriented research can be. It's been a tradition at Rockefeller since its founding, and one of its glories. But I worry," he says, "that, if the present national trend continues, clinical scientists will become an extinct breed. That would be a great shame. Clinical research is not an easy life, it's often frustrating, and you can't



play God with people the way you can with laboratory mice or molecules, but it can also pay big dividends in terms of basic science as well as in practical therapeutic terms."

Clinton Brown, who has just completed a clinical fellowship and, like Dr. Hudgins, plans to continue in research, conducted an experiment in Dr. Ahrens' laboratory with a technique that had never been tried before in the treatment of coronary artery disease. His patient, a man in his mid-fifties, lived for nearly two years solely on intravenous feeding, what is called total parenteral nutrition (TPN). "It takes a lot of motivation to live without food in the mouth," says Dr. Brown, "and Jack has it. He's had two heart attacks and his mother died of heart disease. He's not a suitable candidate for bypass surgery, and when he came to us his angina was so bad he couldn't work."

TPN is conventionally used with people who have lost part of their intestinal tract through injury or surgery. "There is very little literature on cholesterol metabolism in these patients," Dr. Ahrens says, "but they don't seem to suffer coronaries. We wondered if we could use TPN not just to slow Jack's cholesterol buildup, but perhaps also to make it regress."

Dr. Brown put his patient on a regimen in which the substance used to hold together the fat emulsion in the TPN diet was phospholipid, a lipid with phosphorus attached, which also travels in the blood with cholesterol. "We think phospholipid acts as a detergent," says Dr. Brown. "With TPN we were able to bring Jack's synthesis rate down to normal. He feels great. Now we have to find out whether or not the phospholipid has pulled stored cholesterol from his arterial tissue."

Last summer an eleven-year-old could be glimpsed at Rockefeller dancing around to her Walkman and chasing after the laboratory's volleyball team. Her name is Katenia, and she is among several children – the youngest is three – under treatment at the hospital for a rare genetic disorder called familial hypercholesterolemia, or FH.

FH represents a catastrophic failure of the cholesterol transport system. The carriers that take cholesterol from the liver to the cells are called low density lipoproteins (LDL). There are receptor molecules on cell surfaces that "recognize" LDL, attach to it, and escort the LDL-cholesterol complex into the cell. In peo-



Nurse-practitioner Rachael Kolb and Katenia.

ple with FH, the genes that code for the receptor are defective. LDL-cholesterol doesn't get into the cells, so the liver keeps on making cholesterol and copiously turning it out into the blood plasma. Children born with the worst form of the disease, like Katenia, rarely live past their twenties.

When she first came to Dr. Ahrens, Katenia had the telltale lumps of cholesterol in her skin and wildly elevated blood cholesterol levels typical of these children, and she wasn't doing much dancing. After testing, Dr. Ahrens recommended her for a portacaval shunt, an operation that redirects the blood from the intestines around the liver so that the liver makes less cholesterol. In addition, she goes for LDL pheresis every week or two, a procedure in which her blood is pumped out and the plasma portion is passed through a column in which antibodies remove the LDL. LDL pheresis was invented by Willi Stoffel of the University of Cologne, a former member of Dr. Ahrens' laboratory. Dr. Parker learned the procedure from Professor Stoffel and now supervises a joint Rockefeller-New York Hospital team that performs it at New York Hospital.

The lumps in Katenia's skin are gone and her blood cholesterol level is much lower, if not normal. Her status is monitored every six to twelve months with repeat cardiac and metabolic studies in the Rockefeller University Hospital. Whether these interventions will slow the course of her illness only time will tell.

THE DIET DEBATE

FH represents far less than five percent of coronary artery disease. What causes the other 95 percent? Smoking, high blood pressure, and high blood fats can lead to lesions on artery walls that are rich in cholesterol. Recent discoveries about the normal roles of lipoproteins have generated important new understanding of the genetics of their formation, as well as the factors, including diet and exercise, that affect their levels in the blood: too high a level of LDL is believed to contribute to atherosclerosis, while HDL (high density lipoproteins, the ones that transport cholesterol out of tissues), are considered beneficial.

Last January, a report was released showing that in a ten-year trial of a large number of men with high blood cholesterol lev-

els, the rate of heart attacks was reduced when the lipid-lowering drug cholestyramine was administered. The report has been cited as definitive proof of the efficacy of lowering blood levels by *any* means, including diet. But Dr. Ahrens feels that it is "not scientifically sound to extrapolate drug results to advice on diet for people in whom synthesis-absorption feedback regulation works efficiently." For instance, in a recent test of fifty-one patients who were healthy except for high blood cholesterol levels, Dr. McNamara found that seventy-five percent showed *reduction* of cholesterol synthesis on higher cholesterol intake – evidence of good feedback control – and only fifteen percent showed an increase in plasma cholesterol levels. Dr. McNamara points out that low-fat diets lower both LDL and HDL levels, and states that "maintaining a constant ratio of LDL to HDL may in actuality confer no real benefit."

Dr. Ahrens worries that drastic changes in fat consumption, both in quality and quantity, which have been observed to change cell-membrane structure, may have undesirable effects on an individual's immunologic responsiveness and susceptibility to other diseases. He would prefer to see people follow common-sense measures against smoking, obesity, and stress (which raises LDL levels) while maintaining a balanced diet. He urges that the health community direct its attention "to the twenty percent or so of the population at the top end of the blood cholesterol level scale who need vigorous, *individualized* testing and treatment. It's not a popular theme song these days," he says, laughing, "but more and more experts are learning the tune."

While the great debate continues, Dr. Ahrens finds comfort in planting trees in an arboretum that he and his wife started seven years ago in the Catskills. Their more than three hundred species are, he reports, "living happily on a dietary regimen that seems to give rise to no signs of atherosclerosis."

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