

## research progress report

*To summarize in systematic order the reports of progress in arteriosclerosis research supported by grants of the Public Health Service, Dr. Murray Goldstein prepared for the National Advisory Heart Council and for limited distribution a detailed and documented account of the studies and their relation to the pathogenesis, treatment, and prevention of this disease. Dr. Goldstein is an assistant to the chief, Grants and Training Branch, National Heart Institute, Public Health Service.*

*The following paper, drawn from the original report, aims to call attention to the general scope of*

*the research, rather than to evaluate individual findings or to describe them in precise detail. At this stage, with so much still unknown and with many studies still in progress, it is not possible to establish an accurate perspective. Also, this paper excludes much valuable work that is unpublished or is supported by institutions other than the Public Health Service. Compressing the information and arranging it in this context has inevitably resulted in somewhat arbitrary statements and has perhaps strained the intended meaning of the reports by the scientists.*

# Arteriosclerosis Studies

RESEARCH in cardiovascular health is a prime function of the National Heart Institute, a division of the Public Health Service. Since its founding in 1948, in addition to conducting research in Government laboratories, the institute has granted more than \$3.5 million to universities and other institutions to support research related to studies of arteriosclerosis. Most of this amount applies to studies which are still in progress.

The pattern of this research is determined largely by the applicants for grants. The scientists themselves pose the questions which must be answered in advance of further progress in the field.

### Definition and Classification

Relatively few grants have been sought for defining the entity of arteriosclerosis, that condition marked by loss of elasticity, thickening or hardening of the arteries, or atherosclerosis, a specific type of arteriosclerosis characterized by lipoidal and plaque-like changes in the innermost layer of the artery, the intima. One definitive project classifies an entity known as coronary

heart disease, from studies of heart muscle lesions. Correlated clinical findings and electrocardiograms are offered to support this diagnosis.

### Experimental Studies in Pathogenesis

The need to develop experimental techniques is both primary and chronic in the search for contributing factors in arteriosclerosis. The successful use of animals to induce sclerotic processes comparable to those in man began with Anitschkow's work with rabbits in 1908. Since then, experimental studies have employed guinea pigs, rats, dogs, cockerels, chickens, rabbits, and Cebus monkeys. Work supported by the Public Health Service has produced many basic contributions to experimental techniques, involving control of diet, administration of chemicals, action of hormones, transfusion of serums, physical injury, genetic influences, and the effects accompanying aging.

An early need in experimental techniques was to develop simple and reliable methods of inducing the process of arteriosclerosis. Cholesterol, a lipid found in animal fats and oils,

whole milk, egg yolk, liver, and certain forms of seafood, was associated with the sclerotic process by Anitschkow. Since diets high in cholesterol do not invariably produce the condition, other contributing forces have been looked for. Low thyroid activity is one. Arteriosclerosis develops in dogs fed a high cholesterol diet supplemented by thiouracil, a chemical which reduces the production of thyroid hormones. The thiouracil does not so affect dogs on a normal diet.

Readings of cholesterol in the serum of dogs are used in efforts to predict the severity of arteriosclerosis. The injury regresses if the serum cholesterol falls below a certain level. Once the necessary fatty state in serum is established, arteriosclerosis may develop in a matter of days or even hours.

Instead of using thiouracil or thyroidectomy to induce arteriosclerosis in dogs, a laboratory may save time and effort by using radioactive iodine to depress thyroid activity. Curiously enough, the loss of thyroid activity resulting from surgery, followed by an associated rise of cholesterol in the blood, does not produce coronary atherosclerosis in patients with a history of cor pulmonale, a heart disease secondary to disease of the lungs, or rheumatic heart disease.

A process for inducing atherosclerosis for experimental study, without prolonged cholesterol feeding, consists of planting diethylstilbestrol, a synthetic chemical with action resembling a female sex hormone, under the skin of a chicken. This chemical produces a sustained condition of excess lipid in the serum of chickens and, consequently, arterial lesions which closely resemble those found under controlled conditions.

Rats resist efforts to induce arteriosclerosis even when diabetic, although it is common to find atherosclerosis associated with diabetes in humans. On spectral analysis, the fatty proteins in rat serum differ from those of other experimental animals. Rats, which fail to develop a high level of cholesterol in the blood on a fatty diet, rapidly develop such a condition on the identical diet when rabbit serum is transfused into them. This experience suggests that the difference between rat and rabbit plasma may be the basis for the difference in susceptibility of these species to arteriosclerosis.

A study of the relation between physical in-

jury to the artery wall and the formation of atheromatous plaques finds that weanling albino rats are remarkably resistant to the consequences of injury induced by injection of sodium acetylsulfathiazole. The severity of the consequent lesions increases with the age of the rats injured. Injury to the artery wall of rabbits subsequently fed a high cholesterol diet produces a lesion similar to human arteriosclerosis. Of associated interest, microscopic studies reveal hemorrhage within the artery wall in sudden human deaths from coronary artery disease and coronary atheromatosis.

### Fundamental Studies

The driving forces in the arteriosclerotic process may be in the fluids surrounding the artery wall, in the behavior of the cells of the wall itself, in various forces of environment or custom, in genetic influence, or in a combination of several conditions.

#### *The Artery Wall*

Studies of the cellular metabolism of the artery wall itself have been stimulated by the realization that atherosclerosis may occur without the appearance of excess fat in the plasma. One such study demonstrates that the rat artery may convert acetate into fatty acids and can incorporate phosphorus into the phospholipid molecule. The artery wall itself thus can contribute to the production of lipids. To facilitate similar studies, an investigator has developed a technique for measuring the metabolism of intact arterial tissue under aseptic conditions.

Some spontaneous coronary artery lesions of birds are found to be fibrotic rather than fatty; fats may have a late part even in the forming of arteriosclerotic plaques in the aorta of old dogs. Another investigation finds that mucopolysaccharide deposits occur before the lipids. These studies are associated with other efforts to work out the precise cellular history of what appears to be a proliferative and degenerative disease.

The aging factor in the arteries has been associated with the formation of bone or cartilage in the aorta of senile rabbits. Calcification of the media, another sign of aging, is said to precede the formation of intimal plaques, although this

conclusion is not supported by other studies. Autopsies demonstrate that arteriosclerosis increases with age in both sexes. It is more severe, however, in the male. The relatively greater susceptibility in boys appears before their 10th year.

Still other techniques are being developed to pursue the study of the role of the arteries in the hardening process.

### *The Artery's Surroundings*

Studies of the artery's chemical and physical environment deal with three main fields: cholesterol and phospholipids; lipoproteins; and physical forces, including blood stream pressure and turbulence.

The presence of cholesterol and its esters in diets, in plasma, and in arterial plaques associated with arteriosclerosis has commanded particular attention.

The work of identifying, measuring, and exploring the metabolic processing of these lipids deals with the production and destruction of cholesterol, the differentiation between cholesterol taken in the diet and that formed by the body, and the activity of "trace companions" of cholesterol as atherosclerotic agents.

The fate of cholesterol in the intestine is affected by certain micro-organisms and by chemical conditions which may prevent or accelerate its absorption into the blood stream.

The liver is the chief organ for removing excess cholesterol from the blood. It excretes 60 percent of the excess as bile acid. When unknown forces prevent the return of plasma cholesterol to the liver cells, an excess of fat appears in the blood.

Young animals appear to form more cholesterol in the liver than their elders. Low calorie diets reduce the rate of such formation; high calorie diets restore it. The rate is depressed by a high fat diet, which also seems more effective than a low fat diet in removing cholesterol accumulated in the liver of rats. A high level of cholesterol in the blood does not appear in dogs with deficiency of magnesium or in the liver of rats with a deficiency of pantothenic acid. A deficiency of pyridoxine is accompanied by a high level of cholesterol in the blood of *Cebus* monkeys and rats, but not in rabbits. Even with a calorie deficiency, chicks on a high

cholesterol diet have excess cholesterol in the blood and develop atherosclerosis. As calorie intake rises, successive increments of blood cholesterol decrease. Other dietary fats in combination with cholesterol appear to induce higher fat and cholesterol levels in human blood than cholesterol alone.

Detergents, which have a wetting action that keeps fats and oils in suspension so that they do not cling to affected surfaces, increase all blood lipid components, especially phospholipids. An increase in phospholipids appears to retard or prevent atherosclerosis. The ratio of blood cholesterol to blood phospholipid (C/P ratio) may be of importance in retarding atherosclerosis.

Two substances found in the blood stream, known as lipofanogen and antilipofanogen, respectively, support or oppose the forming of visible fat granules in tissue culture. As cholesterol combines with antilipofanogen, the opportunity for lipofanogen to create fat is enhanced.

Obstruction of the bile duct has been associated with high cholesterol levels in the blood. On the assumption that these levels are supported by the action of retained bile salts, it was discovered that cholate impedes the transfer of cholesterol from plasma to liver. High blood cholesterol may be a development secondary to disorder of cholate metabolism.

In addition to occupation, housing, and dietary patterns, genetic factors may be significant in the tendency to form cholesterol.

Cholesterol and phospholipids in blood exist in combination with proteins rather than in a free state. Such combinations are known as lipoproteins. The amount of lipids in such proteins is measurable by a flotation process which permits analysis according to the density of various classes of lipoproteins in plasma. These are classified in terms of Svedberg flotation (Sf) units. Four laboratories engaged in such investigations are participating in a cooperative study on lipoproteins and atherosclerosis.

Efforts to measure the volume and forms of lipoproteins in the blood and in fixed tissues are proceeding with the prospect that these measurements may help to predict the imminence of atherosclerotic change, to diagnose the present degree of atherosclerosis, and to guide the management of the condition and its consequences.

If it is determined what classes of lipoproteins are most formidable, preventive or therapeutic measures may follow.

Because the level of serum cholesterol and lipoprotein has been found to vary in men over a 10-week period or more, single readings may not serve as a reliable indication of a patient's condition.

The liver, one of the organs which produce lipoproteins, increases its output of certain classes if it is chronically inflamed or if it is poisoned by carbon tetrachloride. Hepatectomy causes a gradual decline in all lipoproteins.

Animal species vary in their typical patterns of lipoproteins and in their susceptibility to the effects of different lipoprotein classes. Young women have more alpha lipoprotein fractions (associated with alpha globulin) and fewer beta fractions than young men. Many men and older women tend to have lower levels of albumin and alpha lipoproteins and relatively higher levels of beta lipoproteins than young women. (This information correlates with the greater tendency of men and older women to develop atherosclerosis.) The same tendency appears in patients with clinical signs of atherosclerosis, as well as among persons with diabetes, nephritis, and xanthomatosis, even when their cholesterol or C/P ratio is not elevated. The level of Sf 12-20 beta lipoproteins is higher in persons with diabetes if they are also arteriosclerotic. The concentration of such lipoproteins has been reported to be a more accurate indicator of atherosclerosis than the cholesterol level. In some circumstances, it is this lipoprotein factor which links obesity with atherosclerosis.

Effects of heparin, a normal trace constituent of the blood, are undergoing extensive study in the search for a chemical which may influence the formation of arteriosclerosis.

Physical forces examined in relation to arteriosclerosis include surface tension (detergent action), filtration of fats by artery and capillary walls, turbulence of blood in the aorta of rabbits, and elevated blood pressure (hypertension). Although hypertensive patients show a high incidence of arteriosclerosis, the experimental evidence so far does not establish that the one condition necessarily induces the other.

## Clinical Studies

There have been no research projects on the incidence of morbidity from arteriosclerosis because of the difficulties of reliable diagnosis.

A reliable method by which the incidence or severity of arteriosclerosis could be measured objectively has been the goal of many investigations. Determinations of serum cholesterol, cholesterol tolerance, lipoprotein fractions, phospholipids, cholesterol-phospholipid ratio, lipofanogen-antilipofanogen ratio, or neutral fat have been suggested as possible indexes of the probability of developing or having already developed arteriosclerosis.

In man, excluding histological study after biopsy or necropsy, the only reliable method of diagnosis at the present time depends on the onset of symptoms associated with a decreased blood supply to specific organs or parts of the body. For better objective evaluation of these observations, many methods and devices have been suggested and are being perfected; some of these are flicker flame photometry, plethysmography, ergometry, electrocardiography, vector cardiography, ballistocardiography, vascular catheterization, roentgenography, and angiography. As yet, no generally acceptable means has been agreed upon for the preclinical diagnosis of arteriosclerosis.

## Prophylaxis and Therapy

Diet, medication, surgery, and rehabilitation are being studied as means of preventing or treating arteriosclerosis.

The administration of adrenal cortical and corticotrophic hormones is associated with high levels of cholesterol and lipids in the serum without an increase in lipoproteins or in atherosclerosis.

The relative susceptibility of males to arteriosclerosis has encouraged studies of the effects of synthetic and natural sex hormones on the occurrence and treatment of this disease.

Studies of thyroid effects find that administration of thiouracil or thyroidectomy does not cause regression of arteriosclerotic lesions in dogs.

After biological experiments indicated that

a portion of the mammalian brain affects the movement of cholesterol into the blood stream, a lipid-poor, cholesterol-free residue of this organ was fed to patients with arteriosclerotic heart disease and a high level of cholesterol in the blood. Their serum cholesterol fell "significantly," presumably as a result of the chemical effect blocking the absorption of sterols in the intestine.

Common "lipotropic agents" necessary for fat metabolism, such as choline, methionine, and inositol, have no beneficial effects on the incidence or severity of arteriosclerosis. Ferric chloride decreases absorption of cholesterol from the alimentary canal but not its production by the liver. Other chemicals which reduce absorption are sitosterol and dihydrocholesterol.

The prospect of prophylaxis by diet is not materially brightened by studies to date. A decline in Sf 12-100 lipoproteins accompanies a loss of weight. The Kempner rice diet reduces serum cholesterol and the C/P ratio. It is not certain that restriction of cholesterol in the diet will ordinarily reach the level needed to produce clinical results. The amount of cholesterol in the diet may vary widely without

producing marked effects on the levels in the blood. Restriction of cholesterol in the diet must be accompanied by considerations of total nutritional needs, such as the part proteins play in lipid metabolism.

Surgery to repair or replace vessels damaged by arteriosclerosis has employed a variety of techniques. These include generation of inter-coronary vessels in a heart inflamed by abrasive asbestos powder; grafting of substitute arteries which have been dehydrated or taken out of cold storage; the use of prosthetics made of metal, plastic, latex, or thorotrast; and the patching of arterial walls with peritoneum. The technique of grafting has evoked studies of optimal length, source, structure, sterilization, and storage of blood vessels.

Studies of rehabilitation of cardiac patients are actively encouraged. Energy requirements, as measured by oxygen consumption, are being measured for various occupational activities.

Most of the clinical studies noted here are undertaken in association with experimental laboratory studies, in order to assure that the patient will have the advantage of validated theories, technical experience, and full understanding of the biochemical factors.

## PHS films

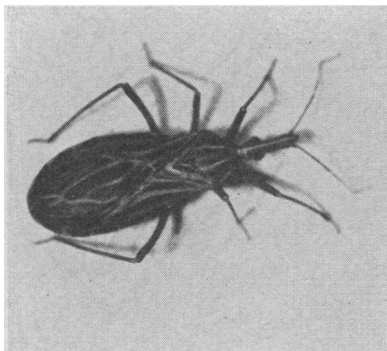
### Arthropods of Public Health Importance

35 mm. filmstrip, sound, color, 7 minutes,  
42 frames. 1954.

Audience: Entomologists, parasitologists,  
sanitarians, and students interested in  
arthropods.

Available: Loan—Public Health Service,  
Communicable Disease Center, 50 7th  
St., NE., Atlanta 5, Ga. Purchase—  
United World Films, Inc., 1445 Park  
Ave., New York 29, N. Y.

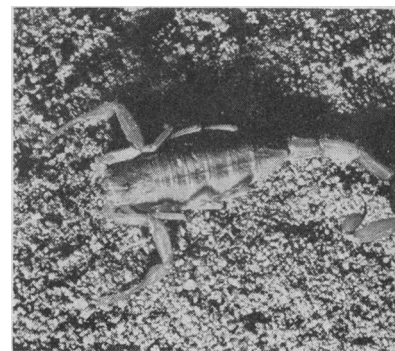
Arthropods of public health significance are featured in this filmstrip.



**Rhodnius prolixas, a kissing bug**

It shows the outstanding characteristics of some of the more common insects and in a few instances gives the life cycle of one or more species of a particular arthropod. Included

are: cockroaches, mosquitoes, flies, bedbugs, *Triatoma*, fleas, lice, scorpions, tarantulas, spiders, centipedes, ticks, and mites.



**Centruroides, a poisonous scorpion from Arizona.**

## Legal Note on public health

## Court Acceptance of Delayed Birth Certificate Filed by Court Order

The recording of a birth certificate was held by the Federal District Court, in the case of *United States v. Casares-Moreno*, 122 F. Supp. 375 (S. D. Cal. 1954), to be only prima facie evidence of its validity, even though it was filed pursuant to an order of the Superior Court of Los Angeles County, and was subject to rebuttal.

The defendant, on trial as an alien who attempted to enter the United States after his deportation, produced a public birth certificate, filed with the California registrar under delayed filing procedures, showing that his parents had given birth to a son in the United States. The birth certificate indicated on its face that it was offered for filing pursuant to order on August 13, 1936, of the Superior Court of Los Angeles County.

The prosecutor, in rebuttal, contended that the son born in the United States was not the defendant but his brother and that the defendant had taken the name and identity of the brother.

The jury found that the defendant was not born in this country.

The defendant did not contest the court's proposition that ordinarily an instrument which the law required to be recorded is only prima facie evidence of the validity of the instrument. He did contend that since the birth certificate in question was recorded pursuant to an order of the superior court it thereby gained some higher status and became a judgment which under the full faith and credit clause of the Constitution had to be treated as a conclusive adjudication of facts stated therein.

The Federal District Court rejected this contention on the following grounds:

"There is no indication that the California Legislature intended to raise records or parts of records so belatedly established to any greater status than the normal registration records which are never, in cases of birth recordation, to be taken as irrebuttable evidence. In other words, it appears that the role of the superior court in ordering the recordation partakes of an administrative function. It is merely an act of recordation which has been permitted by judicial action rather than by an administrative officer. The judgment in such action is not that the facts so found are absolutely conclusive as between petitioner and the rest of the world, but rather, the judgment is that the registrar is ordered to make such a recording."

The court went on to say:

"It is important to note that even if the California Legislature did intend that such a determination was conclusive (which this court finds it did not intend to do) such an intention would, most probably, run afoul of Constitutional prohibitions. Making such an *ex parte* appearance conclusively establish the facts surrounding a person's birth or death might well infringe on and conclusively determine the rights of any third person having contractual or other relations with, and regulatory duties concerning, such person. This would appear to deny such third person due process."

For a similar holding see *Ex parte Lee Fong Fook*, 74 F. Supp. 68 (N. D. Cal. 1948), where the Federal District Court held that jurisdiction to adjudicate citizenship status of a United States resident has never been conferred by Congress on State courts and, consequently, any State court judgment purporting to exercise that jurisdiction cannot, to that extent, claim of the Federal courts full faith and credit.

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Prepared by the Office of the General Counsel,  
Department of Health, Education, and Welfare.

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