AMERICAN HEART ASSOCIATION

COUNCIL ON ARTERIOSCLEROSIS

AMERICAN SOCIETY FOR
THE STUDY OF ARTERIOSCLEROSIS

SEVENTEENTH ANNUAL MEETING

LOS ANGELES, CALIFORNIA

OCTOBER 23-25, 1963

ABSTRACTS

Listed alphabetically by first author

(P) Papers presented at the meeting

† Established Investigator, American Heart Association, 1962-1963
‡ Research Fellow, American Heart Association, 1962-1963

Certain abstracts for papers presented on Friday, October 25, are included in
Abstracts of the American Heart Association Scientific Sessions

A complete program of all events is printed separately
AHA COUNCIL ON ARTERIOSCLEROSIS

AMERICAN SOCIETY FOR THE STUDY OF ARTERIOSCLEROSIS

17th Annual Meeting

ABSTRACTS

Effect of Methyltestosterone on the Serum Cholesterol Pattern of Dogs Maintained on Semisynthetic Diets (P)

Liese L. Abell and Erwin H. Mosbach, New York, N. Y.

The addition of fat to a diet containing 1 per cent cholesterol abolishes the hypocholesteremic effect of methyltestosterone in dogs. The effect of altering the relation between the major constituents of the diet (protein, fat, carbohydrate) upon the activity of this drug has been further studied in cholesterol-fed dogs. In synthetic diets containing casein, lard, salt mixture, vitamins, 1 per cent cholestrol, and either sucrose or cornstarch, the amounts of fat or protein were varied at the expense of carbohydrate.

Groups of 5 dogs were maintained on each given regimen for 4 weeks, and the cholesterol content of the α and β lipoprotein fractions was determined at regular intervals. Methyltestosterone (200 mg. per day) was fed to each dog during pertinent periods. It was found that increasing the per cent of calories derived from lard (5, 30, 45, 55 per cent) enhanced the hypercholesteremia resulting from cholesterol feeding. Under these conditions, methyltestosterone lowered the cholesterol concentration of the α lipoprotein fraction by about 50 per cent, but increased the cholesterol content of the β lipoprotein fraction proportionally, so that the serum total cholesterol concentrations remained unchanged. However, when the protein content of the high cholesterol diet was lowered from 19 to 9 per cent of the calories, methyltestosterone reduced the serum cholesterol levels from an average of 387 to an average of 217 mg. per cent (P < 0.01). The cholesterol concentrations of both α and β lipoprotein fractions fell in the same ratio.

Effect of Estrogens and Androgens on the α- and β-Lipoprotein Composition in Human Subjects (P)


The increase in α-lipoprotein concentration in serum of subjects given estrogens is well known. The effect of estrogen on the composition of the lipoprotein macromolecules is virtually unstudied. The β-D 1.006 to 1.063 Gm./ml.) and α-D 1.063 to 1.210 Gm./ml.) lipoproteins were isolated by sequential preparative ultracentrifugation, washed repeatedly to remove contaminating lipoprotein and albumin, and their homogeneity established by starch gel electrophoresis. They were then analyzed for cholesterol, phospholipids, and protein.

Three hypogonadal women were studied before and during estrogen administration. Estrogen administration markedly diminished the ratio of cholesterol/phospholipid and cholesterol/protein in both α- and β-lipoprotein. The ratio of phosphorus/protein was only slightly diminished in α-, unchanged in β-lipoprotein. The anticipated increase in the amount of serum lipid present as α-lipoprotein, with little change in the amount of serum lipid present as β-lipoprotein, occurred during estrogen administration in all subjects, although the amount of cholesterol present in the α- and β-lipoprotein complexes per unit weight of phospholipid or protein diminished.

The data are consistent with the thesis that the basic "carrier" in both α- and β-lipoproteins is a phospholipid-protein complex capable of combining with cholesterol and additional phospholipid. This thesis is also supported by quantitative analysis of very high-density lipoproteins (D > 1.210 Gm./ml.) which contain only protein and phospholipid. Estrogens increase the amount of lipids circulating as α-lipoprotein by increasing the number of high-density phospholipid-protein units (D > 1.063 Gm./ml.), probably by stimulating synthesis of the α-lipoprotein protein moiety.

Studies in 3 hypogonadal men given androgens suggest an effect opposite that of estrogen on α-lipoprotein composition.

Electron Microscopy of Livers from Rats and Rabbits Treated with Large Doses of Nicotinic Acid

Rudolf Altschul, Saskatoon, Saskatchewan, Canada

Large doses of nicotinic acid taken for decreas-
ing of blood and tissue cholesterol may cause in humans anomalous liver function tests. These tests become normal again, either without interrupting the treatment or after discontinuing it. Moreover, in the livers of experimental animals, some authors found an increase in fatty acids or in total lipids, whereas other investigators found no such changes. Livers from rats and rabbits treated by us with large doses of niacinic acid for up to 6 months showed no increase in total fat and no pathologic changes under the light microscope. To verify the absence of morphologic changes in a still more accurate way, we treated 3 adult rats with 0.04 Gm./day of niacinic acid 6 times per week for 2, 3, and 5 weeks respectively, and 6 adult rabbits with 0.6 Gm./day 6 times weekly for 5, 5, 6, 6, 7, and 8 months respectively. Liver tissue taken after these periods by laparotomy during anesthesia, and examined under electron microscopes Philips 75 and 100B, showed neither anomalies nor pathologic changes.

Effects of 3, 5-Diodothyroacetic and 3,3',5-Triiodothyroacetic Acids on the Time Course of Steroid-C\(^{14}\) Metabolism in the Rat (P)


Adult female Wistar strain rats were maintained on a diet containing cholesterol-4-C\(^{14}\) to effect elevation of blood and liver cholesterol concentrations and labeling of extrahepatic cholesterol pools. After 3 weeks, the animals were divided into equal groups and fed the following diets: (a) control; (b) diiodothyroacetic acid supplemented (5 mg./100 Gm.); (c) triiodothyroacetic acid supplemented (5 mg./100 Gm.). Rats were placed in individual metabolism cages and feces, urine, and carbon dioxide collected daily for 2 weeks. For tissue studies, rats were sacrificed before and after the regression period. Di- and triiodothyroacetic acids had similar effects: rapid increases in mobilization rates of serum and liver cholesterol-4-C\(^{14}\); and smaller increases in rates of extrahepatic \(\beta\) sterol-C\(^{14}\) mobilization. Both acids doubled the excretion rate of fecal \(\alpha + \beta\) sterol-C\(^{14}\), but neither affected the rate of fecal bile acid-C\(^{14}\) excretion. Urinary-C\(^{14}\) excretion was moderately elevated in the treated rats. These changes in steroid-C\(^{14}\) excretion were not altered when after 5 days there was an increase in carbon dioxide production. No carbon-C\(^{14}\) dioxide was expired by any of the rats at any time. The results show that, in the rat, di- and triiodothyroacetic acids increase the elimination of tissue cholesterol via fecal \(\alpha + \beta\) sterol—and to a lesser extent via urinary steroids—but do not increase sterol side-chain oxidation.

Selective Blood Lipid Reductions by Newer Pharmacologic Agents

Donald Berkovitz, Philadelphia, Pa.

Although the precise significance of an elevation of any of the various blood lipid fractions is not known, sufficient circumstantial evidence is at hand to inerminate hyperlipidemia in the pathogenesis of atherosclerosis, either casually or causally. Accordingly, intensive efforts are being made to prepare pharmacologic agents which possess specific blood lipid lowering properties.

A group of hypercholesteremic patients with and without an accompanying hypertriglyceridemia have been treated with several of these new lipopenic preparations. Administration of a bile sequestering resin was found to effectively lower the serum cholesterol with no effect on the triglyceride levels. A phospholipid preparation, rich in polyunsaturated fatty acids, appeared to be capable of lowering the serum triglycerides with little effect on the cholesterol, whereas treatment with a mixture of androsterone with ethyl-p-chlorophenoxyisobutyrate resulted in a significant decrease in both the cholesterol and triglyceride levels. The radioactive character of the resin was typical of those patients who demonstrated a significant decrease in their triglycerides. Normalization of the blood lipids was frequently accompanied by steatorrhea when the resin was used, but did not occur with the phospholipid or androsterone combination.

The data reported indicate that it is possible to selectively decrease either the cholesterol or the triglycerides, or both, by pharmacologic agents.

Interrelationship of Dietary Protein, Sulfur-containing Amino Acids and Fats on the Plasma Cholesterol Levels in Men and Women (P)

James M. R. Beveridge, W. Ford Connell, and Shih-Chen Hu, Kingston, Ontario, Canada

Ninety-one university students, 50 males and 41 females, were divided into 2 groups, A and B. Group A (25 males and 20 females) consumed for 8 days a homogenous formula diet providing 30 per cent of calories from butterfat and 15 per cent from preparations of milk proteins. They were then divided into 2 subgroups of equal size and continued for another 8 days on a diet low in protein (5 per cent of calories), with and without the amounts of methionine and cystine supplied by the additional protein in the first ration. The subjects in group B (25 males and 21 females)
were placed on a similar homogeneous formula diet providing 30 per cent of calories from corn oil. The plasma cholesterol level of the male subjects on the low-protein diet containing butterfat and without supplements showed a significant increase at the end of 8 days (+13.0 mg./100 ml., \( P < 0.01 \)). In the case of the female subjects, the increase noted was not significant (+3.4 mg./100 ml., \( P > 0.05 \)). No significant increase was observed in those with supplements of methionine and cystine for both male and female subjects (+2.1 mg., +2.0 mg./100 ml., respectively). No significant changes were found in the plasma cholesterol levels in the men and women of group B, both with and without the sulfur-containing amino acid supplements. The changes in mg. cholesterol/100 ml. from day 8 to 16 were in the case of no supplements: male, +0.7 mg., female —4.0 mg.; and with supplements: male, —0.2 mg., female, —3.8 mg. A hypothesis will be advanced to explain the difference in response to the supplementation with the sulfur-containing amino acids of the low-protein diets containing butterfat on the one hand, and corn oil on the other. No explanation can be offered as yet to the difference in response noted for the 2 sexes.

Complete Postmortem Evaluation of Cerebral Circulation


Methods for complete postmortem evaluation of cerebral circulation have been developed. These methods include evaluation of clinical data for evidence of contributory extracerebral complications such as shock or anemia. General postmortem findings are also evaluated and include careful study of the aorta and heart. Arteries of the neck (carotid and vertebral arteries) are analyzed by a method developed in our laboratory. The circle of Willis is studied. Anterior, middle, and posterior cerebral arteries are studied by washing with saline at 100 mm. Hg, followed by injection of a colored, roentgen-opaque gelatin preparation. Intrinsie cerebral arteries are studied by x-ray and by clearing techniques. Techniques for clearing one-half of a cerebral hemisphere have been developed. The perfused intrinsic arteries are clearly visible in deep areas in the cleared brains. These methods should be of great value in the study of intrinsic arterial lesions which precede and lead to cerebral thrombosis and hemorrhage. Good microscopic sections of selected, cleared brain, and arterial tissue can be prepared and studied.

The experimental group in the study is confined to deceased patients over 50 years old; consideration of wasting diseases such as diffuse carcinomatosis is being evaluated in the study. Patients dying between the ages of 1 and 50 years have been selected as controls. A search for possible increased collateral flow in the principal cerebral arteries of older individuals (with severe disease of major cerebral arteries) is being made.

Localization of Cholesterol Absorption

Henry Buchwald, Minneapolis, Minn.

Through a standardized experimental procedure, this study attempts to determine the intrinsic abilities of the upper and lower small intestine to absorb cholesterol, and the fundamental differences in their respective capacities. Six-month-old New Zealand white rabbits were divided into 3 groups of 10 rabbits (5 male and 5 female), representing (1) normal controls, (2) rabbits with the upper half of their small intestine and, to insure equal transit time, their ileocecal valve bypassed, and (3) rabbits with the lower half of their small intestine bypassed. Each rabbit was fed 10 mg. of cholesterol-4-C\textsuperscript{14} (0.4 \mu c./mg.) in 2 ml. of Mazola oil. Absorption was measured by the average 6-day whole-blood radioactivity.

By halving the available small intestinal absorptive surface and decreasing transit time, an extremely large lowering of cholesterol absorption was observed: an 84.5 per cent reduction (2,417 counts/min., controls; 374 counts/min., bypassed) of the normal mean absorptive capacity in the ileal bypass group, and an 80.9 per cent (2,417 counts/min., control; 461 counts/min., bypassed) reduction in the jejunal and ileocecal valve bypass group. Whole-blood cholesterol was also significantly lower in both bypass groups: 28 per cent lower in the ileal bypass group (91.8 mg. per cent, controls; 66.1 mg. per cent, bypassed), and 15.6 per cent lower in the jejunal bypass group, (91.8 mg. per cent, controls; 77.5 mg. per cent, bypassed).

In conclusion, both the upper and lower small intestine are capable of absorbing cholesterol; however, there appears to be a somewhat greater absorptive ability in the lower intestine, i.e., ileum rather than duodenum-jejunum.

Surgical Operation to Lower Circulating Cholesterol (P)

Henry Buchwald, Minneapolis, Minn.

To effect substantial lowering of the absorption of cholesterol excreted into the intestinal tract, as
well as of exogenous cholesterol, and thus to lower blood levels significantly, a therapeutic operation, a one-half terminal ileum bypass (about 200 cm.), is proposed. This procedure would not interfere with normal protein and carbohydrate absorption. Bypass is preferable to excision because it is simpler, is reversible, and permits the bypassed loop to continue to excrete cholesterol.

Ten rabbits subjected to bypass of the distal half of their small intestine were force-fed 10 mg. cholesterol-C\(_{14}\) (0.4 \(\mu\)c./mg.), 10 to 27 days postoperatively, as was a matched control group. The operated group had 85 per cent less absorption, as measured by whole-blood radioactivity (2,417 counts/min., controls; 374 counts/min., operated), and 28 per cent less whole-blood cholesterol (92 mg. per cent, controls; 66 mg. per cent, operated). Eight pigs serving as their own preoperative controls, after bypass of only the distal one-third of their small intestine, presented essentially identical results.

Blood samples taken after feeding 50 mg. cholesterol-C\(_{14}\) (0.2 \(\mu\)c./mg.) to 7 human patients with an average resection of 96 cm. terminal ileum (performed for strangulated hernia, etc.), and to an age- and sex-matched group of controls, showed 36 per cent less absorption, as measured by plasma radioactivity (386 counts/min., controls; 248 counts/min., operated), and 26 per cent less plasma cholesterol (183 mg. per cent, controls; 136 mg. per cent, operated) in the resected portion. Notably, the patient with the lowest resection, 180 cm., showed virtually no cholesterol absorption.

A surgical program for hypercholesteremic patients has been started at the University of Minnesota Hospitals.

Proposed Prediction Index for Myocardial Infarction and Cerebrovascular Accident


Relationships found among blood serum components represented in lipoproteins have been used in developing a proposed prediction method for myocardial infarction and cerebrovascular accident (CVA). Serum from 137 human male subjects distributed in 4 categories was studied: (a) myocardial infarction or CVA history; (b) symptoms without history; (c) age controls, 45 years or older; and (d) young adult controls, 18 to 22 years. Nine variables were determined before and after 48-hour serum incubation at 37 to 38 C.

Most incubation changes have direction associated with myocardial infarction or CVA history; each is quantitatively different and a possible factor in total change. Subjects with history have larger than control mean values for beta-lipoprotein cholesterol, triglycerides, phospholipids, \(\alpha_1\)-, \(\alpha_2\)-, and beta-globulin (group 1); smaller for gamma-globulin, albumin, and alpha-lipoprotein cholesterol (group 2). Proposed prediction index is based upon direction and relative amount of incubation change. Product of group 1 incubated/nonincubated ratios divided by product of group 2 ratios measures total change. This value times 100 equals the Prediction Index.

Mean indices for (a), (b), (c), (d) are 171, 171, 137, and 127 respectively. Standard deviations are 70, 70, 79, and 59. Individual indices are interpreted as more severe when larger than 1 standard deviation below mean for (a), >101; no indication when less than corresponding value for (d), <68; less severe for intermediate indices, 101 to 68.

Results are in accord with known category (a) history. Distribution in all categories and other observations suggest value of this and related approaches to prediction studies.

Rapid Development of Atheroma


Pathological studies of human aortas in populations at different ages suggest that the evolution of atheroma, although variable in rate of development, usually proceeds as a gradual process over a 20- to 30-year period. Serial observations of the atherogenic process in vivo are possible with modern angiographic techniques. These have disclosed that atheromatous lesions, at times, develop much more rapidly than formerly assumed.

Seven patients with hypertension and atherosclerotic renal arterial stenosis had deferment of their operation for a number of reasons. A repeat renal arteriogram done 1 to 3 years later demonstrated variable rates of progression of observed lesions. There were 9 stenotic renal arterial lesions at first arteriography in this group of patients, and 13 lesions at the second arteriography. Angiographic grading was estimated by the Sutton method.

Four new lesions obliterating about one-half or more of the diameter of the lumen (grade III) developed within less than 3 years. Two grade III lesions (about three-fourths of the diameter of the lumen obliterated) progressed to grade IV (total or almost total occlusion) in 2 years. Four lesions showed no progression. In 1 patient a grade II lesion of the left renal artery progressed slightly over a 1-year period, and the patient exhibited a
new development of a grade III stenosis of the right renal artery in that 1-year period.

The variability and generally more rapid development of these lesions suggests a modification of the traditional concepts of the rate of atheroma formation in man.

Scrum Lipids, Enzymes, and Vigorous Exercise

George L. Calvy, Camp Le Jeune, N. C., Menard M. Gertler, and Lee D. Cady, New York, N. Y.

The relationship between exercise and a high-calorie, high-saturated-fat diet was studied over a 22-week period in 101 Marine trainees with an average of 20.5 years. Their diet consisted of 4,500 calories (fat 2,025 calories, carbohydrates 2,025 calories, and proteins 450 calories). The program consisted of 16 hours of rigorous daily activity. There were no statistically significant changes in uric acid (4.8 mg. per cent before training and 5.0 mg. per cent after training), lipid phosphorus (8.2 mg. per cent before training and 9.4 mg. per cent after training), total cholesterol (176 mg. per cent before training and 183 mg. per cent after training), lactic dehydrogenase (244 units per cent before training and 231 units per cent after training), malic dehydrogenase (72 units per cent before training and 91 units per cent after training). Weight (162 pounds before training and 165 pounds after training) and blood pressure (118/71 before training and 118/78 after training) did not change. Serum content of triglycerides rose significantly (42 ± 3 mg. per cent before training and 91 ± 5 mg. per cent after training); isoecitrill dehydrogenase dropped significantly (6.6 ± 0.3 units before training and 3.4 ± 0.3 units after training). It is suggested that this high-calorie and high-saturated-fat diet (milk, butter, and eggs) may not be atherosclerogenic if sufficient calories are utilized to offset this intake. These facts and observations have been essentially confirmed in a similar group of 137 Marine trainees, average age 18.5 years.

Regulation of Plasma Arachidonic Acid by the Pancreas (P)

Raymond Caren and Lucille M. Corbo, Los Angeles, Calif.

This study demonstrated the presence of a heat-stable factor in canine external pancreatic secretion that regulates the plasma arachidonic acid. It can be supplied by feeding fresh pork pancreas, and is distinct from the pancreatic enzymes.

Studies were done comparing the fatty acids of unfractonated plasma, cholesterol ester, phospholipid, triglyceride, and unesterified fatty acid fractions by gas-liquid chromatography of 4 depancreatized and 5 pancreatic duct ligated dogs before and during feeding of pancreatic enzyme preparations. Following removal of the exocrine secretion in both groups, the per cent composition of arachidonic acid fell to at least one-half of the control value (average 15.8 per cent) in approximately 2 weeks while oleic acid rose. Oral administration of purified lipase and whole-gland concentrate for 25 to 58 days caused oleic acid to fall to near control values, but arachidonic acid remained one-half of the control value. The major concentration of arachidonic acid was in the cholesterol ester and phospholipid fractions. The changes in unfractonated plasma were reflections of the changes in these two fractions. There were no significant changes in any of the other fatty acids.

Fresh pork pancreas restored arachidonic acid to normal, or above, in unfractonated plasma. This was due to elevation of arachidonic acid in the cholesterol ester and phospholipid fractions. The triglyceride and unesterified fatty acid fractions were not significantly affected. Boiled fresh pancreas was ineffective, but produced the same results as the unboiled material when fed with purified lipase and whole-gland concentrate.

Trans-1,4-bis-(2-chlorobenzylaminomethyl) cyclohexane dihydrochloride (AY 9944): A Novel Inhibitor of Cholesterol Biosynthesis (P)

Clifford L. Chappel, Dusan Dvornik, Peter Hill, Michael Kraml, and Roger Gaudry, Montreal, Canada

The effect of trans-1,4-bis (2-chlorobenzylaminomethyl) cyclohexane dihydrochloride (AY 9944) on the lipid metabolism has been studied. In vitro, at a final concentration of 1 × 10^-3 M, AY 9944 inhibited the incorporation of mevalonate-2-C^14 in liver homogenates of rats (82 per cent), dogs (21 per cent), and monkeys (59 per cent). In rats, 1 single oral dose of 10 μM/Kg. (4.64 mg./Kg.) of AY 9944 depressed the hepatic cholesterol synthesis 92 per cent after 2 hours, 72 per cent after 24 hours, and 55 per cent after 48 hours respectively. In vitro, at a final concentration of 1 × 10^-3 M, AY 9944 did not significantly influence the incorporation of mevalonate-2-C^14 into squaleine or lanosterol, nor did it affect the conversion of 24-dehydrocholesterol to cholesterol. In normal rats, 10 μM/Kg./day of AY 9944 given orally for 7 days reduced serum sterols from 93 mg. per cent to 24 mg. per cent. Continued treatment did not cause further reduction. Two weeks after treatment was discontinued, both hepatic cholesterol synthesis capacity and serum sterol levels re-
turned to normal. In the rat, the lowest daily oral dose of AY 9944 which after 7 days of treatment produced a significant depression of serum sterols was 2 \( \mu \text{M/Kg.} \) for females and 3 \( \mu \text{M/Kg.} \) for males. When given as a single oral dose, the median lethal dose of AY 9944 in rats was 1.163 \( \mu \text{M/Kg.} \). Examination of blood and tissues of animals treated with AY 9944 revealed the presence of a “fast-acting” steroid which was isolated and identified as 7-dehydrocholesterol peroxide. Application of differential spectroscopy analysis indicated that treatment of normal male rats with AY 9944 (10\( \mu \text{M/Kg./day orally for 7 days} \) produced in the serum sterol levels consisting of 17 mg. per cent cholesterol, 14 mg. per cent 7-dehydrocholesterol, and 3.5 mg. per cent of another as yet unidentified “fast-acting” sterol. Thus, AY 9944 appears to prevent the conversion of 7-dehydrocholesterol to cholesterol.

Further Observations on Lipid Synthesis in Human Blood Vessels

Aram V. Chobanian and William Hollander, Boston, Mass.

Recent in vitro studies in this laboratory have indicated that the human arterial intima can actively take up, synthesize, and catabolize long-chain fatty acids. The present investigation was undertaken to examine further the utilization of fatty acids by the arterial wall and to continue to study the local synthesis of lipids by the intima. Segments of human arteries removed at surgery were incubated with C\(^{14}\)-acetate and albumin-bound C\(^{14}\)-labeled palmitic, stearic, oleic, and linoleic acids. Separation of the arterial lipid classes was carried out by thin-layer chromatography, and isolation and identification of the fatty acids by gas chromatography. The results indicate: (1) Much of the labeled fatty acids taken up by the intima from the incubating medium is recovered from the arterial wall in the unesterified form. However, significant amounts of the entering fatty acids are incorporated into the intimal phospholipid and triglyceride moieties. (2) Little or no incorporation by the intima of fatty acids into cholesterol esters is apparent. (3) Significant intimal synthesis of phospholipids and triglycerides, as well as of free fatty acids, from acetate is evident. (4) Most of the acetate utilized for lipid synthesis is incorporated into the free fatty acids and phospholipids, with lesser amounts into the triglyceride fraction. The addition of glucose to the incubation medium increases the relative rate of triglyceride synthesis. These in vitro studies indicate that the human intima can synthesize phospholipids and triglycerides from acetate and can incorporate unesterified fatty acids into arterial phospholipids and triglycerides.

Coronary Heart Disease and Hypertension in the White Mountain Apache Tribe (P)

Nathan J. Clifford, Ventura, Calif., John J. Kelly, Jr., San Diego, Calif., Thomas F. Leo, Hempstead, N. Y., and Howard A. Eder, Bronx, N. Y.

Clinical experience at the U.S. Public Health Service Indian Hospital at Whiteriver, Arizona has been that coronary artery disease is very rare among the Apaches. In 147 electrocardiograms recorded on individuals above the age of 40, not a single instance of myocardial infarction, left bundle-branch block, or second- or third-degree atrioventricular block was disclosed.

The mean serum cholesterol in men above the age of 20 was 198 ± 42 mg./100 ml. and in women 205 ± 41 mg./100 ml.; 86 per cent of Apache men aged 45 to 64 had cholesterol levels below 225 mg./100 ml. as compared to 72 per cent of those aged 25 to 44. Cholesterol was determined in 188 individuals.

A survey of food purchases by 6 representative families, comprising 33 individuals and 2,413 subject-days, revealed that their average daily calorie intake was 1,465 calories (both children and adults). Of this, 10.2 per cent were supplied by protein and 24.1 per cent by fat. Dairy foods were rarely consumed.

Elevated blood pressure was found to be remarkably common. The prevalence among men was unusually high, particularly among the younger age groups, with 48 per cent of those aged 30 to 39 having blood pressures which reached, or exceeded, 160/95 mm. Hg. Diastolic pressures of 100 mm. Hg or above were recorded in 26.4 per cent of the men and 23.2 per cent of the women, out of a total of 327 individuals.

Lipids, Purines, Glucose, and Heredity in the Younger Coronary Disease Patient

Burt Cochran, Jr., and Edward P. Marbach, Los Angeles, Calif.

Clinical coronary artery disease appearing between the ages of 24 and 45 years was studied in this group of 25 otherwise unselected adult subjects. Both hospital and clinic followup were used to obtain a minimum of 3 basal blood specimens for fasting glucose, total cholesterol, total lipid, lipid phosphorus, free fatty acids, uric acid and protein-bound iodine. Many subjects have been followed for up to 3 years in order to evaluate spontaneous fluctuations of these biochemical pa-
rameters. Subjects also received a test meal or standard glucose tolerance test, and plasma and subcutaneous tissue fatty acid composition was studied by gas-liquid chromatography under standardized dietary conditions. The 10 women exhibited more frequent obesity (7 subjects, including 3 with weights of 270 to 348 pounds), diabetes mellitus (3 frankly diabetic, 1 borderline, and a total of 6 with a diabetic parent or sibling) and premature coronary attacks in the family (4 subjects). Contrasted with the 15 male subjects, they had less hyperuricemia, diastolic hypertension, xanthonata, and lower mean circulating cholesterol (276 mg, per cent), coupled with less lability of serum cholesterol levels (fluctuations usually under 60 mg per cent). In addition, the ratio of relative proportions of serum linoic and palmitic acids in the female subjects was somewhat more depressed (1.08 versus a normal of 1.63) than was that of the males (1.21 versus normal 1.61). Both serum and tissue fatty acids were not specifically altered, with the exception of a slight relative increase (certain 35 per cent) in palmitoleic acid in depot fat samples.

Isozymes of Lactic Dehydrogenase (LDH) Following Myocardial Infarction (P)

Louis Cohen, Juliana Obradovich, and Vadim Orniste, Chicago, Ill.

Lactic dehydrogenase exists in 5 electrophoretically distinguishable forms: LDH 1, 2, 3, 4, and 5. Organs rich in 1 or more of these isozymes appear to contribute LDH to the serum when injured. It remains to be proved that specific organ injury can be detected by the appearance of a characteristic serum isozyme pattern.

The serum LDH in 40 healthy subjects was 285 ± 85 units/ml. LDH 1, 2, and 3 respectively have 37, 48 and 15 per cent of this activity. LDH 4 and 5 are occasionally normally evident, but display only a few per cent of the total activity.

Isozyme studies were performed in over 150 patients. The serum LDH increase in disease results from an increase in the activity of 1 or more of the isozymes. A tremendous and characteristic increase in LDH 1, but also LDH 2, occurred in 20 cases of myocardial infarction. This abnormal pattern of LDH 1 > LDH 2 after infarction persisted several days after the total serum LDH returned to normal; ultimately, the normal relationship of LDH 2 > LDH 1 became reestablished. LDH 1 > LDH 2 also developed following coronary insufficiency, even though serial assays of total LDH activity remained within normal limits. Of the many conditions studied to date, only the isozymic pattern in per-

nicious anemia mimicked that of myocardial infarction. These isozymic shifts should be of especial aid in the diagnosis of a recent infarction, both when the total LDH is high or has recently returned to the normal range.

Regression of Cholesterol-4-C14 from the Blood and Tissues of Animals: Effects of Dietary Corn Oil Compared with a Low-Fat Diet (P)

William E. Connor and Carl S. Jackson, Iowa City, Iowa

The body pool of cholesterol in rabbits was labeled with cholesterol-4-C14 given 1 μc. weekly by gavage. Concurrently, 15 rabbits received a diet containing 0.5 per cent cholesterol plus 2.5 per cent peanut oil. After 14 weeks, the feeding of cholesterol-4-C14 and 0.5 per cent cholesterol was stopped, and tissues of 5 animals were analyzed for cholesterol content and radioactivity. For the next 14 weeks, 18 per cent corn oil was given to 5 animals, while 5 others received only the low-fat Purina® chow.

Cholesterol feeding produced comparable serum cholesterol levels (1,792 to 1,993 mg. per cent) in all groups. Serum specific activities (Sp.A.) reached 453 to 483 (counts per minute per mg. of cholesterol). Regression of serum cholesterol was to 382 mg. per cent for corn oil (C.O.), and to 88 for low fat (L.F.). Serum Sp.A. declined to 312 (C.O.) and 200 (L.F.). Liver cholesterol fell from 142 mg./Gm. to 88 (C.O.) and 19 (L.F.). Aortic cholesterol increased from 87 mg./Gm. to 121 (C.O.), and 91 (L.F.). Liver Sp.A. changed from 437 to 319 (C.O.), and 261 (L.F.). Aortic Sp.A. did not decrease: 348, 346 (C.O.) and 379 (L.F.), nor did aortic atherosclerosis. Sp.A. in xanthoma, eye, adrenal, intestine, spleen, kidney declined slightly, more so for the L.F. group. Biliary cholesterol and Sp.A. remained higher in C.O. animals.

Corn oil treatment did not increase the removal of cholesterol from tissues and atheromatous lesions. Cholesterol equilibrated very slowly in both atheromatous and normal aortas. Hypercholesterolemia persisted because cholesterol moved from tissues to blood.

Rapid Production of Atherosclerosis in the Rat

Paris Constantinides, Vancouver, Canada

It is well known that the rat is unusually resistant to the development of true atherosclerosis (with intimal proliferation and foam cells) in response to chronic lipemia alone. Attempts to increase atherogenesis in this species by combining chronic lipemia with systemic ar-
terial injury have so far yielded rather disappointing results, largely because the vasotoxic agents employed in such experiments usually cause extensive medial necrosis and lipoidosis as well as myocardial necrosis and, therefore, a very high mortality. We have recently found that one can produce within 6 weeks true intimal foam cell lesions in rat arteries (particularly coronaries)—with negligible myocardial damage and mortality—if one induces some lipemia during the first 2 weeks with the help of a cholesterol-thiouracil-cholate diet, insults the arteries for only 3 days with viosterol during the third week, and continues the same lipemia-producing diet for another 3 weeks before killing. It appears that the main reason for the success of this particular procedure was the fact that by first adapting the rats to relatively harmless steroids such as cholesterol and bile acids, we increased their general resistance to a much more toxic steroid such as viosterol—thus causing minimal arterial injury and permitting maximal intimal hyperplasia and lipohagia.

Coagulation Incidental to Stress

Herbert L. Davis, Omaha, Nebr.

Studies of stress in humans and in dogs reveal that there is a general tendency to hypercoagulability. In humans in childbirth, in surgery, and in physical exercise, the reduced clot times may be correlated with elevated concentrations of free fatty acids. In dogs, the coagulant effect of anesthetics complicates the interpretation, but the ionic fatty acids are often elevated in burns, hemorrhage, asphyxiation, or electric shock, and the clot times in siliconed tubes are generally reduced to 2 minutes or less. Other coagulant mechanisms such as acidosis (carbon dioxide) appear to be involved.

So marked are these coagulant effects in humans that whole-blood clot times in glass as low as 2 minutes have been observed, and in many instances blood from dogs in acute or terminal stages of stress clotted in spite of normally effective concentrations of heparin or of oxalate. These findings suggest that intravascular clotting depletes the fibrinogen store, and may thus lead to hemorrhagic episodes. Recognition of such mechanisms may lead to effective remedial measures and thus minimize clotting to death.

On the Mechanism of Serum-induced Thrombosis (P)

Daniel Deykin and Stanford Wessler, Boston, Mass.

Human glass-contacted serum infused into rabbits induces stasis thrombi similar to red thrombi observed in man. The activity in human serum initiating these thrombi has been termed serum thrombotic accelerator (STA) activity. Silicome-processed normal serum as well as glass-contacted serum from patients deficient in coagulation Factors XII (Hageman), XI (PTA), or IX (PTC) develop little STA activity. In contrast, glass-contacted serum from patients with other clotting defects develops normal STA activity. Activated Factor XI (Activation Product, AP), isolated from normal or Factor IX-deficient blood, has also been shown to have thrombus-inducing activity.

In view of these data, glass-contacted serum from normal and Factor IX-deficient subjects was tested for AP and STA activity. Whereas 29 of 30 infusions of normal serum generated full STA activity, only 8 of 36 infusions of Factor IX-deficient serum yielded comparable STA activity. The AP content of the Factor IX-deficient serum overlapped the normal range. The lack of STA activity in Factor IX-deficient serum did not correlate with its AP content. These observations demonstrate that, under conditions of glass-contact activation, it is the absence of Factor IX activity, rather than the level of AP, that limits the elaboration of STA activity. Since both heparin and dicumarol interfere with the formation of activated Factor IX, the data cited have implications concerning the antithrombotic action of these anticoagulant drugs.

Total Body Cholesterol of the Rat; Response to Cholesterol Feeding and to Alteration of Thyroid State

Charles H. Duncan, Maurice M. Best, and Ronald J. Lubbe, Louisville, Ky.

To evaluate the effect of thyroid state on cholesterol content and distribution in the rat, 4 groups of 3 animals were studied. Group I served as control, group II and III were made hyperthyroid by daily injection of 0.04 μM L-thyroxin (L-T4) or 0.20 μM D-thyroxin (D-T4) respectively, and group IV was surgically thyroidectomized. At the end of 4 weeks, all rats were exsanguinated and the heart, lungs, liver, kidneys, spleen, brain, gastrointestinal tract, thymus, testes, and adrenal glands removed and individually weighed. The carcass was dissected into depot fat, skin, and the combined muscular supportive tissues. The cholesterol concentration of each of the various organs and tissues was determined. The mean total rat cholesterol concentration (in mg./Gm.) was: control, 1.72; L-T4, 1.91; D-T4, 1.90; and thyroidectomized, 1.71. The increase in total body cholesterol concentration of the 2 hyperthyroid groups was due largely to an increased concentration
in the skin (from 4.07 to 4.68 and 4.44 mg./Gm., respectively) and to hypertrophy of the viscera with relatively high cholesterol concentrations.

Four additional groups of rats, similarly studied except that 1 per cent cholesterol was added to the diet, yielded the following total rat cholesterol concentrations: control, 2.25; L-T4, 2.06; D-T4, 1.97; and thyroidectomized, 2.40 mg./Gm. Cholesterol feeding resulted in increased cholesterol concentrations in most tissues, notably the liver (1.61 to 12.16). The lower total concentrations in the hyperthyroid groups were due entirely to lower liver concentrations (3.12 and 3.14 mg./Gm. respectively).

DOCA Hypertension in Contrast to Renal Hypertension in the Etiology of Coronary Atherosclerosis in Adult Meat-fed Male Wistar Rats (P)


Renal hypertension in meat-fed rats has been found to produce hypercholesterolemia and lipid deposition with characteristic alterations of coronary arteries observed in atherosclerosis. To investigate another cause of hypertension under similar dietary conditions, 53.4 mg. desoxycorticosterone acetate (DOCA) was implanted subcutaneously between the shoulders of adult male Wistar rats. Blood pressures were taken before and periodically after implantation with a photelastic tensiometer. Ground beef, fortified with vitamins and minerals, was fed twice daily in slight excess. At sacrifice, serum and tissue cholesterol were determined by the Abell method. Heart and aortas were examined histologically by Oil Red O procedures. Of 45 DOCA rats sacrificed 8 to 48 weeks after implantation, 2 (48 weeks) had hypercholesterolemia, but none had lipid deposition in coronary arteries. At 16 weeks, left ventricular hypertrophy was observed, but the coronary arteries were normal through 30 weeks. However, at 39 weeks, subendocardial fibrosis, hyalinosis of the musculature, patchy intramural fibrosis with medial hypertrophy and thickening of the coronary artery walls accompanied by hyalinization and loss of nuclei had occurred. The aortas were always normal. Four rats died of unknown causes; 2 (19, 38 weeks) had slight medial lipid infiltration in 1 coronary branch. In contrast, renal hypertensive (Grollman) rats developed hypercholesterolemia and coronary lipid deposition as early as 13 weeks after operation; 19 of 26 rat hearts (taken 14 to 24 weeks after operation) had lipid in coronary artery walls and 14 of these 19 were hypercholesterolemic. (No serum cholesterol was obtained from 3 that died).

Obviously, renal hypertension is more atherogenic than DOCA hypertension in meat-fed rats.

Cholesterol Excretion by Renal Hypertensive and Normal Rats Fed Chow and Meat

Charles H. Eades, Jr., Victoria B. Solberg, Irene C. Hsu, Carol A. Wallhausen, and George E. Phillips, Morris Plains, N. J.

Meat-fed renal hypertensive (Grollman type) adult male Wistar rats usually develop hypercholesterolemia from 14 to 16 weeks after operation. Similar chow-fed rats are not so consistent but also may develop hypercholesterolemia with time (20 to 27 weeks). Since the 2 diets have the same cholesterol content (126 mg. per cent, dry) but differ in fat (chow 4 per cent; meat 46 per cent) and protein (chow 23 per cent; meat 46 per cent), it was of interest to determine their effects on fecal cholesterol (Liebermann Burchard positive sterols calculated as cholesterol) in rats under study from 20 to 33 weeks. Feces from comparable groups (I, normal chow-fed; II, normal meat-fed; III, hypertensive chow-fed; and IV, hypertensive, meat-fed) were collected for 1 week, lyophilized, weighed, and ground to a powder in a Wiley mill.

Samples were saponified, the cholesterol extracted with petroleum ether, and aliquots analyzed for cholesterol. Results show that all groups excreted more cholesterol than was consumed in the diet; chow-fed rats ate more and excreted more cholesterol (total amount) than the meat-fed rats; hypertensive rats consumed and excreted slightly more cholesterol than the normal counterparts; and the hypertensive meat-fed group excreted the greatest percentage excess over intake of all groups. Furthermore, the hypertensive rats were hypercholesterolemic (above 100 mg. per cent), whereas the unoperated rats were normal. These data suggest that an increased cholesterol biosynthesis by chow-fed and meat-fed renal hypertensive rats has developed, and that the extra fat in the meat diet of the hypertensive rat may enhance the cholesterol loss in the feces.

Effect of Polybrene on Fasting Serum Triglycerides in Man

Hyman Engelberg, Beverly Hills, Calif.

Polybrene is an anti-heparin agent which has no effect on blood coagulability in unheparinized individuals. It partially inhibits the lipolytic and clearing activity of lipoprotein lipase. Accordingly,
polybrene was given intravenously over a 2-hour period in postabsorptive patients, either in 5 per cent glucose or in normal saline.

In 7 of the 16 patients who received polybrene in glucose, and in 12 of the 15 who were given polybrene in normal saline, the serum triglycerides rose from 10 to 50 per cent over the 2-hour period of the infusion. These results indicate that lipoprotein lipase in the intravascular compartment, that is, in the plasma or at the capillary endothelium, functions in the removal of endogenously synthesized triglyceride from the bloodstream in man.

Effect of P/S Ratio, Partial Hydrogenation of Dietary Fats, and Dietary Cholesterol upon Plasma Cholesterol in Man (P)


The effects on the plasma cholesterol level of dietary cholesterol and of the following fats were determined in humans fed formula diets: Fat A, a partially hydrogenated soybean oil (P/S = 1.6); Fat B, a blend of unhydrogenated vegetable oils (P/S = 1.5) similar in composition to Fat A; Fat C, a mixture (P/S = 0.7) composed of Fat A and cocoa butter; Fat D, a mixture (P/S = 0.7) composed of Fat B and cocoa butter; and cocoa butter (P/S = 0.1). Fats A and B were fed with and without dietary cholesterol provided by dried egg yolk. Fatty acids provided by egg yolk or by an equal quantity of a control fat simulating egg yolk triglycerides were included in calculating P/S ratios. Fat supplied 41 per cent of the total calories. Forty-two subjects were divided into 7 groups and fed the formula diets for 4 periods of 5 weeks each. Each group received a different dietary fat during each period, and blood samples were obtained twice weekly throughout the study.

Plasma cholesterol levels were unaffected when the P/S ratio of the dietary fat was varied from 1.6 to 0.1 in a cholesterol-free diet. The cholesterol response to the partially hydrogenated fat was identical to that of the unhydrogenated fat of similar composition. Addition of cholesterol (742 mg. daily) to diets containing Fats A and B increased plasma cholesterol levels 23.8 (192.8 to 216.6) and 28.6 (188.3 to 214.9) mg. per cent, respectively (P = 0.05).

Prevention of Spontaneous Atherosclerosis (with Iridium192) in the Cholesterol-fed Rabbit

Meyer Friedman and Sanford O. Byers, San Francisco, Calif.

An earlier study revealed that the anti-atherogenic effect of cortisone administration was due to its suppression of the hyperplastic element in the atherosclerotic process. Therefore, the possible effect of irradiation with iridium192 upon atherosclerosis was studied.

The first 21 cm. of the aorta of 16 rabbits were exposed to a wire containing iridium192 (41.2 to 47.3 mEq. ra./wire) inserted therein for 24 to 48 hours. Two weeks later the animals were given a cholesterol-oil enhanced diet for 6 weeks and then sacrificed. Fourteen control animals exposed to a "cold" wire were similarly fed and sacrificed.

It was found that, despite the parity of serum cholesterol in the 2 groups, profound inhibition of atherosclerosis had been accomplished in the 14 surviving experimental animals. Thus their gross degree of aortic atherosclerosis was 0.5 (controls: 3.5), and their average aortic cholesterol concentration was 2.7 Gm. per cent (controls: 5.7 Gm. per cent). Histologic studies confirmed the grossly observed anti-atherogenic effect of the prior exposure to local irradiation.

Cellular Metabolic Units in Atheromatous Lesions

I. Ernest Gonzalez, San Francisco, Calif.

In the past, the possible significance of foam cells in human and experimental atheromatous lesions has not been clearly defined. We think that hydrolytic enzymes are important and distinctive constituents of atheromatous lesions, and postulate that the breakdown and concentration of some lipids in the intima is related closely to the activity of the foam cells laden with phosphatases, esterases, lipases and other hydrolytic enzymes. As complex lipids are degraded by the enzymes, the soluble amino acids and phospholipids diffuse through the arterial wall. Some triglycerides and fatty acids may be partly resolved to water and CO2. The stable and insoluble cholesterol molecule does not become degraded and concentrates in the intima and forms part of the grumous residue of atheromatous lesions.

Our studies of leukocytes, cultured in millipore chambers located in subcutaneous tissues of cholesterolemic rabbits, that evolve to foam cells suggest that blood monocytes may become foam cells virtually indistinguishable from those found in atheromatous lesions in the same animals. Theoretically, then, a blood monocyte may possibly attach itself along with platelets to damaged or intact endothelial surfaces and penetrate into the subendothelial space. Under the proper environment and stimulus, i.e., hyperlipemia, the monocyte may become a foam cell within an edematous and thickened intima.
Effect of Hyperlipemia on the Thickened Arterial Intima (P)


Since many think that passive imbibition of lipid into an already thickened intima leads to the formation of atheromatous lesions, we have studied cellular, phagocytic and histozeugmatic changes in experimentally induced thickened arterial intimas in hyperlipemic animals. For this observation, air was injected daily into the ear vein of normal rabbits. After 5 to 6 months of air injections, there was marked diffuse proliferation of the intima in the pulmonary arterial bed.

Rabbits with established diffuse fibrous pulmonary arterial lesions were then fed 1 Gm. of cholesterol daily. After 4 to 5 months of cholesterol feedings, 1 or 2 animals were sacrificed at biweekly and monthly intervals. Histochemical studies revealed 2 distinctive lesions. In the arteries with diffuse intimal fibrosis, the diffuse fibrous lesions contained virtually no lipids or hydrolytic enzymes. However, the uninvolved pulmonary arteries in the same animals developed distinctive, focal, eccentric atheromatous lesions containing abundant lipids and high concentrations of hydrolytic enzymes.

These studies suggest that diffuse intimal fibrosis does not potentiate the formation of atheromatous lesions containing high concentrations of lipid and hydrolytic enzymes. We are now studying the effects of hyperlipemia on intimas thickened by focal radiation.

Effect of Norepinephrine Infusion on Plasma Lipids in Normal and Hyperlipidemic Subjects

Sebastiano Grasso, George D. Michaels, Marjorie Coelho, and Launorce W. Kinsell, Oakland, Calif.

It has been well demonstrated that norepinephrine causes a release of free fatty acids from adipose tissue with a resultant rise in plasma lipids. In the present study, norepinephrine was infused at a constant rate over periods of 4 to 8 hours in normal and abnormal subjects, and the levels of plasma glycerides, cholesterol, phospholipids, and free fatty acids measured. In normal subjects, the free fatty acids increased and remained elevated during the entire infusion, but there was little or no change in the levels of other lipids. However, there is some evidence which suggests that mobilization occurs, despite the lack of glyceride elevation; i.e., change in fatty acid pattern of glyceride has been observed. The hyperlipidemic subjects studied included individuals with primary hyperglycieridemia, primary hypercholesterolemia, biliary cirrhosis, diabetes, and some individuals with elevated lipids in association with vascular disease. In most of these individuals, in addition to the elevation of the free fatty acids, very significant increases of glycerides, cholesterol, and phospholipids were observed during the norepinephrine infusion. Also, very significant changes occurred in the fatty acid composition of the different plasma lipids. From these findings, it appears that norepinephrine mobilizes lipoproteins from the liver in many individuals with disturbed lipid metabolism. It is not improbable that the same statement applies to normal subjects, but that the rate of glyceride removal is sufficiently rapid to prevent a net rise in plasma glycerides.

Sex Hormone Influence on Intimal Morphology

M. E. Groover, Jr., Oklahoma City, Okla.

Recent interest in the use of estrogens in preventing recurrent myocardial infarction in man poses the question: Do sex hormones influence the morphology of the coronary intima? The intimal thickness was measured in rats treated with ethinyl estradiol and testosterone cyclopentylpropionate. One group received daily injections of 0.3 ml. cottonseed oil containing none, 0.001, 0.01 and 0.1 mg. ethinyl estradiol. The other group was injected at 3-week intervals with 0.3 ml. cottonseed oil containing none, 0.5, and 5 mg. testosterone cyclopentylpropionate. Each dose level was administered to 12 animals, 1 of which was sacrificed after 4 weeks and at 2-week intervals thereafter. The hearts were fixed by intra-arterial infusion of 4 per cent formalin under constant pressure. Random labeled, tri-chrome stained, histological preparations showing cross sections of coronary vessels were projected on a desktop horizontal screen from which intimal measurements were made. Data were evaluated by the analysis of variance showing the influence and interaction between rat age, hormone dose, treatment time, and hormone used. These studies show: (1) Testosterone and the duration of treatment are synergistic in increasing intimal width regardless of dose level or animal age (significant to the one-hundredth level). (2) Estrogen produces increased intimal thickening with increasing dose level, but not related to duration of treatment (significant to the one-hundredth level). (3) There appears to be an age-estrogen interaction, with thickening in the young and thinning in the old.

Neurogenic Myocardial Necrosis and Protective Effects of Atropine (P)

M. E. Groover, Jr. and Clarke Stout, Oklahoma City, Okla.

In the past myocardial lesions produced by vagal
stimulation were not considered biologically significant because stimuli used exceeded physiological limits. Recently myocardial necrosis produced by stimuli of minimal intensity and duration seems more realistically akin to the clinical attack in man precipitated by emotional tension. In order to determine the relative protective effect of atropine in this condition, graded doses of atropine sulphate ranging from zero to 0.6 mg. were given to 16 African baboons divided into 6 treatment groups. Using sterile technic, the right vagus nerve was exposed and stimulated 20 minutes after atropine administration. The current and frequency were increased until perceptible cardiac slowing was observed on the monitoring electrocardiogram. Allowing 1 minute recovery, the procedure was repeated 12 times. Three weeks later the animals were sacrificed by exsanguination under pentobarbital anesthesia. The heart was sectioned into thin, horizontal slices from apex to base and the slices incubated at 37°C for 20 minutes in a solution of blue tetrazolium at pH 7.4. Myocardial scarring appeared as unstained streaky areas, while the normal myoecardium was stained a deep purple. Myocardial lesions were found in all 3 of the animals receiving no atropine, 1 of 3 receiving 0.05 mg., and 1 receiving 0.1 mg. No myocardial lesions were seen in the remaining 11 animals. Interestingly, fatty streaks appeared on the lesser curvature of the aortic arch in 2 non-atropinized animals and in 1 receiving 0.05 mg. Myocardial necrosis and fatty streaks seem to be related to vagal activity.

Serum and Tissue Lipid Studies in Experimental Dog and Rabbit Atherosclerosis (P)


Dogs and rabbits were fed "normal" diets for each species, supplemented with cholesterol (1 per cent by wt.), thiourea (100 mg./Kg. body weight), or both, to relate serum and tissue lipid changes to arterial lesions. Serum lipids were determined gravimetrically after fractionation by silicic acid column chromatography; liver and aorta total lipid were determined but not further fractionated. The fatty acid composition of each fraction (including depot fat) was determined by gas-liquid chromatography.

Arterial lesions were found only in dogs fed diets supplemented with cholesterol-thiourea, and lesions were present in rabbits fed cholesterol or cholesterol-thiourea. Two changes in lipid content were associated with the presence of arterial lesions. One was a marked, absolute increase in serum cholesterol; the other was a marked decrease in arachidonic acid content of serum cholesterol esters, serum free fatty acids, serum phospholipids, liver lipids, and aorta lipids. Since the metabolic precursor of arachidonic acid, linoleic acid, was present in normal amounts in serum and tissues of both species, there was no dietary or acquired deficiency of linoleic acid. The magnitude of serum cholesterol increase and the presence of arterial lesions were inversely proportional to the level of arachidonic acid in the serum and tissue lipids. It is proposed, on the basis of the serum and tissue content of linoleic acid and arachidonic acid in normal dogs and rabbits, that the difference in response to cholesterol and thiourea between the 2 species is due to a difference in their ability to synthesize arachidonic from linoleic acid.

Role of Serum Copper in Coronary Atherosclerosis (P)

Denham Harman, Omaha, Nebr.

Molecular oxygen has been postulated to react with serum lipoprotein constituents and arterial wall lipid deposits to form products that may be involved in the initiation and development of atherosclerosis. The oxidation products—peroxides and substances formed by oxidative polymerization of polyunsaturated fatty acids and their esters would be expected among these—may produce their effects by acting as tissue irritants.

If oxidation of lipids plays a significant role in the development of atherosclerosis, then the serum concentration of copper, a good oxidation catalyst, might be elevated in individuals with clinical disease. This possibility was studied by determining the copper concentration of fasting serum obtained from employed white male volunteers in the age range 35 to 55 with (30) and without (95) a history of myocardial infarction. The average age and serum copper level in the individuals with a history of myocardial infarction were 46.8 years and 138 ± 26 µg. per 100 ml.; corresponding values for the controls were 43.5 years and 123 ± 22 µg. per 100 ml. The difference between the copper means is significant at P < 0.01.

This positive correlation between serum copper levels and clinical disease supports the possibility that lipid oxidation is significantly involved in atherosclerosis development. In addition, the data suggest that serum copper concentration determinations may aid in identifying coronary-prone individuals (13 per cent of the controls had concentrations above 140 µg./100 ml.), and that lowering of elevated copper levels by dietary or chemical means may decrease the probability of development of clinical coronary atherosclerosis.
Incorporation of Labeled Leucine into Lipoprotein Protein by Rat Intestinal Mucosa (P)

Frederick T. Hatch,† Lillian M. Hagopian, Joel J. Rubenstein, and George P. Canellos, Boston, Mass.

Fasted rats weighing over 300 Gm. were fed 4 ml. olive oil by gavage. One and one-half hours later (during active fat absorption), the rats were sacrificed by ether anesthesia and a blow on the head. The mucosal cells were extruded from the duodenum and jejunum by scraping along the serosal surface. This whole-cell preparation was incubated in Krebs-Ringer bicarbonate buffer or rat serum under a gas phase of 95 per cent O₂-5 per cent CO₂ with the addition of glucose, penicillin, streptomycin, and leucine (labeled uniformly or in the carboxyl position). After 2½ hours of incubation, the lipoproteins were fractionated by differential ultracentrifugation, precipitated with trichloracetic acid, isolated upon Millipore filters, and counted in a liquid scintillation system.

Of 10 million counts/min, added, from 100 to 500 were present in the isolated lipoproteins. Counts in experimental vessels exceeded those in controls with label added after incubation by 5- to 20-fold. Leucine was incorporated chiefly into the following fractions: chylomicrons (density 1.005, ½ hour at 26,000 × g); very low density (density 1.005, 1½ hours at 110,000 × g); and residue (sedimenting at density 1.21). The specific activity of the chylomycin fraction substantially exceeded that of the residual protein. The system was inhibited almost completely by puromycin (.001 M) and 2, 4-dinitrophenol (2 × 10⁻⁴ M).

Technical difficulties were encountered—in particular a temperature-dependent nonenzymatic binding of leucine to lipoproteins.

A limited incorporation of amino acid into lipoprotein protein has been demonstrated with intestinal mucosa cells which were isolated during absorption of fat.

Morphogenesis of Naturally Occurring Arteriosclerotic Lesions in the Pig's Aorta and the Alterations Resulting from Butter Feeding (P)


A total of 87 focal lesions from aortae of pigs ranging in age from one-half to 7-1/4/years were examined. Approximately half of these were removed from pigs fed butter-rich diet for 12 months; the others came from controls. The lesions from the latter were in the gross white or grayish-white range, and varied in size from a dot to elevations measuring 1 cm. They were more numerous in older pigs and in the abdominal aorta. Longitudinal, white long ridges were present in abdominal aorta only. Microscopically, they consisted of fibrous connective tissue and smooth muscle cells (SMC), both in varying degrees of maturation. A young lesion was made up of acid-mucopolysaccharide-rich ground substance, delicate connective tissue (CT) fibers, and numerous young SMC. Old lesions were composed largely of dense collagen. The SMC were fewer and atrophic and often contained fat vacuoles, and some extracellular fat was seen at the base of a large lesion. Often, a lesion was composed of 2 or more layers of CT and SMC's, each layer exhibiting a different degree of maturation, the least mature being richest in proliferating SMC, and subendothelial in position. This superficial layer frequently had tinctorial properties of blood proteins and little if any formed CT elements. This was interpreted as a focal insulation of blood proteins. Focal lesions in the experimental animals were similar to those described in controls but contained more intracellular and extracellular fat. In addition, small thrombi were superimposed upon the lesions. This would support the theory that "encrustation" also played a role in the genesis of these lesions. The morphogenesis of these lesions in the pig is strikingly similar to that of arteriosclerotic lesions in man.

Observations on Some Histochemically Demonstrable Enzyme Activities in Nonarteriosclerotic and Arteriosclerotic Beagle Aorta (P)

F. H. Higginsbotham, Morgantown, West Va., Maurice Sandler, Atlanta, Ga., A. C. Higginsbotham, Morgantown, West Va., and Geoffrey H. Bourne, Atlanta, Ga.

Litters of F₂'s from sibbed F₁ purebred beagles are being used to study morphological and histochemical changes preceding and accompanying experimental aortic arteriosclerosis. Preliminary histochemical studies of a nonarteriosclerotic F₁ have shown circumferential and longitudinal gradients of aortic ATPase activity. Decreased activity is apparent in the subendothelium and inner third of the media of the ascending, arch, upper descending, and abdominal segments. Endothelium and outer media show uniform activity throughout. There are indications that some areas of decreased activity coincide with morphological differences in the aortic root and arch.

In the aortas of F₂'s fed a cholesterol-oil diet
and thiouracil, there is accentuation of the ATPase variability, with spotty focal loss of activity in the ascending, arch, and abdominal segments extending into the middle third of the media. Activity in the outer media and endothelium appears unaffected. Lipid-laden macrophages were seen in widened interlamellar muscle-free spaces in the ascending aorta and arch, and sometimes a diffuse sudanophilia of the inner portion of the wall.

Aortas of F₂'s fed a regular diet and thiouracil also show some decrease of ATPase activity, especially in areas of intimal hyperplasia, but no sudanophilia was observed.

In contrast to rats, cats, and human beings, no 5'-nucleotidase activity was demonstrable in dog aorta.

These findings suggest that reduction in activity of certain histochemically demonstrable enzymes precedes the morphological manifestations of arteriosclerosis.

**Thrombosis Associated with Mobilization of Fatty Acids (P)**

*John C. Hoak, John C. F. Poole, and Donald S. Robinson, Oxford, England*

Long-chain saturated fatty acids (C₁₄-C₂₂) produce generalized thrombosis and sudden death when injected intravenously into animals. Pulmonary edema and congestion are associated autopsy findings. Electron microscopy of these thrombi revealed large aggregates of degranulated platelets.

In the present study, attempts were made to produce thrombosis in rabbits by subcutaneous injections of ACTH and anterior pituitary extract. Two hours after injection, either produced a 4- to 7-fold rise in plasma free fatty acids (FFA). Mean values: before, 595 μg./L.; after, 2,529 μg./L. A jugular vein segment was clamped 2 hours after injecting ACTH gel (50 units/Kg.) or anterior pituitary extract (5 mL/Kg.) and examined for thrombi 15 minutes later. The animals were then heparinized, killed with pentobarbital, and autopsied.

Six of 10 rabbits given ACTH, and 2 of 5 given pituitary extract, developed jugular thrombi. None was found in 5 control rabbits. Pulmonary thrombi were also found in 9 of 15 given ACTH or pituitary extract. Seven additional rabbits were injected with ACTH or pituitary extract. Three died within 24 hours and at autopsy, generalized thrombosis, pulmonary edema, and congestion were found.

Whole-blood silicone clotting times were performed on 5 rabbits before receiving ACTH (mean 41.4 ± 6.2 minutes) and 2 hours after (mean 21.9 ± 6.1). (Controls: before saline, mean 44.4 ± 2.4; after saline, mean 45.0 ± 5.2). *P* < 0.001.

These findings suggest that rapid lipid mobilization resulting in high plasma FFA can be associated with a thrombotic state. Similar mechanisms could be important in the pathogenesis of thromboembolic complications seen in clinical conditions associated with high plasma FFA.

**Composition and Synthesis of Lipoproteins in Human Atherosclerotic Tissue (P)**

*William Hollander, Boston, Mass.*

The present study was undertaken because previous studies indicated that cholesterol in the nonprotein bound form accumulated in arterial plaques and was nontransportable and inflammatory in contrast to cholesterol in the lipoprotein form. Fresh atherosclerotic tissue was removed at surgery for vascular disease and incubated with C¹⁴-acetate. Following incubation, the tissue lipoproteins were extracted in saline and separated ultracentrifugally into low-density (D < 1.019, D 1.019 to 1.063) and high-density (D 1.063 to 1.210) lipoprotein fractions. The saline extracts contained 10 times more low-density lipoproteins than high-density lipoproteins. Over 85 per cent of the extracted cholesterol, triglycerides, and phospholipids were recovered in the low-density lipoproteins, which contained 5 times more lipid/mg. of protein than the high-density lipoproteins. The incubated tissue converted C¹⁴-acetate into the protein as well as the lipid moieties of the low- and high-density lipoproteins. However, 6 per cent of the total lipids and 20 per cent of the total sterols synthesized by the tissues were recovered in the nonprotein bound form. The incorporation of C¹⁴-acetate into the low-density lipoprotein moieties was greater than that into the high-density lipoprotein moieties. However, the specific activities of the high-density lipoprotein fractions were higher than those of the low-density lipoprotein fractions.

Conclusion: The lipoproteins contained and synthesized in atherosclerotic tissue are mainly low-density lipoproteins. A failure of the diseased artery to complex all its synthesized lipids to lipoproteins may account for the nonprotein bound lipid recovered in the tissue.

**Effects of Vasopressor Agents on the Synthesis of Mucopolysaccharides and Lipids by the Arterial Wall**

*William Hollander, Boston, Mass.*

The in vitro effects of epinephrine, norepinephrine and angiotensin on arterial wall metab-
olism were studied because of the possible role of these vasopressor agents in atherosclerosis. After incubating segments of dog aorta in lactated Ringer's solution, the acid mucopolysaccharides were extracted with alkali, precipitated with Cetavolon and isolated with ethanol-sodium acetate. Aortic tissue incubated with epinephrine or norepinephrine in 0.1 to 25 μg./ml converted significantly less S^{35}-sulfate, C^{14}-acetate, and C^{14}-glucose into acid mucopolysaccharides than the incubated control tissue. These differences occurred even though the specific activity of the sulfate precursor was not altered by the catecholamines. Epinephrine also produced more than a 50 per cent reduction in mucopolysaccharide formation in incubation studies with human aortic tissue removed at surgery for vascular disease. The conversion of C^{14}-acetate and C^{14}-glucose to lipids was also studied to determine whether the catecholamines might reduce mucopolysaccharide synthesis by diverting acetate and glucose to other metabolic pathways. The incorporation of acetate and glucose into fatty acids and sterols by dog aortic tissue also was found to be reduced by more than 50 per cent below control values by the catecholamines. Angiotensin in 25 μg./ml. had no significant effect on the synthesis of acid mucopolysaccharides and lipids by the arterial wall.

Conclusion: The synthesis of acid mucopolysaccharides and lipids by the arterial wall is inhibited by the catecholamines, but not by angiotensin.

**Effects of Ultraviolet Irradiation on Skin, Liver, and Serum Sterols and on Serum Triglycerides in the Rat**

*Louis Horlick, Saskatoon, Saskatchewan, Canada*

Rats fed a standard diet alone, or supplemented with 1 per cent cholesterol in 5 per cent corn oil, were irradiated with a medium pressure mercury vapor lamp for short intervals over an 8- to 16-day period. Skin and liver samples were incubated for 2 hours at 37 °C. with 2-H^3-acetate and C^{14}-mevalonic acid. The sterols underwent preliminary separation by thin-layer chromatography (TLC) and, subsequently, further separation and quantification by gas liquid chromatography (GLC). The irradiated rat skin contained more total sterols per gram than did the skin from nonirradiated animals (4.24 mg./Gm. ± 0.5 vs 2.95 ± 0.5, P = .02). The percentage distribution of the various sterols, as determined by GLC, showed no significant change between the groups except for an increase in 7-dehydrocholesterol in the irradiated group. The latter group also incorporated more radioactivity both from 2-H^3-acetate and C^{14}-mevalonate into the sterols than did the control group, but the distribution of radioactivity among the different fractions was no different from the controls.

Total radioactivity from both isotopes incorporated into liver sterols showed no significant differences between irradiated and nonirradiated animals.

Serum cholesterol and triglyceride levels were lower in the irradiated, cholesterol-fed group than in their nonirradiated controls. The adrenal glands of the irradiated animals were grossly hypertrophied.

Ultraviolet irradiation acted as a stimulus to cellular hyperplasia and lipid synthesis in the rat skin, but had no demonstrable effects on the liver. Serum lipids were reduced, but this was considered to be a nonspecific stress effect due to irradiation.

**Effects of Chlorophenoxyisobutyrate and of Chlorophenoxyisobutyrate Plus Androsterone on Serum Lipids and Ultracentrifugally Determined Lipoproteins (P)**

*R. Palmer Howard, Petar Atanovic, and Robert H. Furman, Oklahoma City, Okla.*

Chlorophenoxyisobutyrate (CPIB), or CPIB-A with 1 mg. androsterone per 50 mg. (CPIB-A), was administered orally to 24 subjects. Most were outpatients on diets maintaining weight and serum lipid levels. Every 2 weeks serum was obtained for cholesterol, lipid phosphorus and triglyceride concentrations and lipoproteins by differential ultracentrifugation. After lipid levels were stable, 1.5 to 2 Gm. daily of CPIB-A or CPIB were given for periods up to 4 months. In 10 subjects CPIB was given after the return of serum lipids to control levels following withdrawal of CPIB-A.

In 5 of 6 hyperglyceridemic subjects treated with either CPIB or CPIB-A, marked reduction of serum lipids and very low density lipoproteins occurred. One subject failed to respond, In most of 7 subjects with primary hypercholesterolemia, a moderate decrease in serum cholesterol and β-lipoproteins occurred. In both conditions, women appeared more responsive than men. Decrease in triglycerides occurred in most subjects whose initial triglyceride values were normal. In a hypercholesterolemia-hyperphospholipidemic subject with biliary cirrhosis, serum cholesterol and phospholipid concentrations were reduced to approximately two-thirds of control levels. In a man with methyltestosterone-induced hypercholesterolemia, the serum cholesterol and β-lipoprotein levels were reduced to one-half when either CPIB or CPIB-A was administered while methyltestosterone was continued.
Three men were given 3 to 4 Gm. daily of CPIB-A or CPIB for 2 weeks during metabolic studies in hospital. No anabolic effect was apparent. One developed decreased serum cholesterol esterification and increased transaminase and triglyceride levels.

It is concluded that CPIB has hypoglycemic and hypcholesterolemic effects and that the androsterone in CPIB-A is of no importance.

Effect of Various Fats on Hypercholesterolemia and Atherogenesis in Cockerels

Savitri Jain, Ruth Pick,† and Louis N. Katz, Chicago, Ill.

Previous studies in this department showed no difference in blood cholesterol levels or in incidence or severity of aortic and coronary atherosclerosis in cockerels fed regular mash and cholesterol with various vegetable and animal fats (at the 5 to 10 per cent level) for 5 weeks. To eliminate the possible masking effect on atherogenesis of the 3 to 5 per cent natural fats of unknown composition present in regular mash, in the present study the experiments were repeated after substituting a fat-free semisynthetic diet for the regular mash previously employed. Ten per cent each of a special brand of margarine (with a 1.5 to 1.0 ratio of polysaturated to saturated fats), corn oil, cottonseed oil, butter or coconut oil were added to this diet, as well as 20 per cent protein and 1 per cent cholesterol. These 5 diets were fed for 5 weeks to groups of cockerels 8 to 11 weeks old. Blood and bile cholesterol levels and the incidence and severity of aortic and coronary atherosclerosis were determined. The various fats caused all of these parameters to rise significantly; the magnitude of the effect of the various fats increased in the order listed above. These results suggest that an approximately optimum mixture of saturated and unsaturated fats is less hypercholesterolemic and atherogenic in cockerels than either class of fats given alone.

Factors Influencing Fat Tolerance Curve

Richard J. Jones and Ljubica Dobrilovic, Chicago, Ill.

Fat tolerance curves, derived from the plasma optical density (O.D.) at 0, 3, 4, and 6 hours after a standard 30 Gm. fat breakfast, were performed 62 times in 5 patients. Dairy fat or corn oil were used alternately as the test fat in each patient while diets high or low in fat were given for periods of 2 weeks. There was a delay in the peak rise of optical density when corn oil replaced dairy fat. This was perceptible on the high-fat diet and grossly evident on the low-fat regimen when the peak occurred at 6 instead of 4 hours. This delay could not be attributed to a delay in gastric emptying.

The O.D. was compared with cholesterol, phospholipid, and fatty acid concentrations of lipoprotein fractions separated at densities of 1.006, 1.019 and 1.063. None of the parameters measured at densities >1.006, nor even the cholesterol concentration of lipoproteins of density <1.006 (chylomiera), showed changes paralleling the O.D. The fatty acid and, to a lesser extent, the phospholipid concentration of the chylomieron fraction did, however, vary with the O.D. Unexplained variations in the curve occurred occasionally whether O.D. or chylomieron fatty acids were employed. Although the O.D. provides a fairly reliable indication of the postprandial changes in chylomieron fatty acid concentrations, the shape of the fat tolerance curve is dependent, among other things, upon the previous dietary fat intake, as well as the quality of the test fat.

Role of Age on Experimental Thrombosis in Rabbits

Chizuko Kakita, Ruth Pick,† Philip Johnson, and Louis N. Katz, Chicago, Ill.

One of the significant complications of human atherosclerosis is arterial thrombosis. This report deals with an attempt to gain understanding of the factors involved. For this purpose, normal nonatherosclerotic rabbits were used. Thrombosis was induced by Sawyer’s method of wrapping an abdominal aorta segment with autologous, mechanically injured skeletal muscle. Thrombi were produced by this method within 24 hours. Sham-operated animals served as controls.

Young, immature rabbits showed negative results in both groups. Old, mature rabbits in the experimental group showed a 50 per cent incidence of thrombi compared to an absence of thrombi in the control group. The resistance of the young, immature rabbits and the susceptibility of the old, mature rabbits demonstrate that age is an important factor in thrombus formation. It was found that young rabbits treated with vitamin D preoperatively had thromboses comparable in incidence to the old animals. The mechanism by which vitamin D makes the vascular wall of the young animals susceptible to thrombus formation remains to be elucidated.

These experiments indicate (1) that age is a significant factor in the proneness to thrombogenesis, and (2) that young vessels under certain circumstances, e.g. hypervitaminosis D, can be made to react like older vessels.
Behavior of Factor IX during Oral Anticoagulant Therapy

Francis J. Kazmier, John A. Spittle, Jr., John H. Thompson, Jr., and Charles A. Owen, Jr., Rochester, Minn.

Although 4 clotting factors are depressed by orally administered anticoagulants, namely, VII, IX, X, and prothrombin, the usual test for control of therapy is the prothrombin time, which does not reflect the concentration of factor IX. Since a deficiency of IX can cause serious bleeding (Christmas disease), the validity of prothrombin time as a method for control of anticoagulant therapy has been questioned.

Bishydroxycoumarin, warfarin, phenprocoumon, or phenindione was administered to 11 patients. Dosage was regulated by prothrombin time. With use of specific deficient plasma for the assay of factors VII, IX, and X, and a 2-stage assay for prothrombin, daily levels of these factors were measured during induction and maintenance, and in most cases following cessation, of therapy.

With all drugs, factor VII was uniformly depressed rapidly; it fell to less than 20 per cent within 48 hours after initiation of therapy. Levels of factor X and prothrombin decreased more slowly and did not reach 20 per cent in less than 96 hours. Changes in factor IX activity more closely paralleled those of factor VII, lagging some 24 hours behind during induction and recovery. During maintenance therapy, when levels of factor IX ranged from 5 to 15 per cent, levels of factor VII were in the same range. Four patients on long-term therapy showed the same VII-IX parallelism. Because of this fortuitous relationship between these 2 factors, and despite its insensitivity to IX, prothrombin time appears to be a reliable guide to anticoagulant therapy.

Hypercholesteremic Effect of Nicotine Administration in Dogs


A relationship between cigarette smoking and elevated serum cholesterol has been frequently observed and the question of an etiologic association is being investigated. In the present study, 9 mongrel dogs weighing 8 to 12 Kg. were maintained on a balanced ration. After baseline lipid levels were established, the animals were given daily intramuscular injections of nicotine bitartrate (0.5 to 1.0 mg./Kg.), in a delayed absorption aqueous vehicle containing 2 per cent glycerin and gelatin, for a 6-week period. The mean serum cholesterol level increased gradually from a pre-nicotine value of 144 ± 11.8 mg. per cent to a peak of 216 ± 15.3 mg. per cent (P < 0.01) at the end of the fourth week (50 per cent rise). This approximate level was maintained with nicotine for 2 additional weeks. The drug was then stopped and the cholesterol dropped to 181 ± 13.2 mg. per cent (P < 0.05) after 2 weeks. There was no significant change in serum triglyceride level. Eleven similarly fed and caged control dogs, 5 of which received injections of the vehicle only without nicotine, showed no significant change in cholesterol level. There was no notable change in weight or feeding pattern in the nicotine or control groups. This hypercholesteremic effect of nicotine probably arises in the liver secondary to an increase in circulating catecholamines.

Isolation and Quantitation of Plasma Estrogens in Patients with Hyperlipemia


The purpose of the study is to determine whether hyperlipemic individuals exhibit a qualitative and/or quantitative difference in the estrogens present in their blood when compared to asymptomatic normals. The documented fact that estrogens possess the ability to alter abnormal serum lipid values in the direction of normal, as well as the observed rarity of myocardial infarction in premenopausal women, suggests the implication of the female sex hormones in the genesis of atherosclerotic disease which may be characterized by hyperlipemic states.

The estrogenic hormones were chemically extracted from plasma obtained from asymptomatic normal and hyperlipemic males and females. Isolation and separation were carried out by gas chromatography as described by Kroman and Bender, and Kroman, Bender and Capizzi.

Hyperlipemia was established by determining the concentration of total serum cholesterol, serum triglyceride, and serum alpha- and beta-lipoproteins.

Results indicate that 92.6 per cent (12 cases) of those individuals having normal concentrations of blood lipids (asymptomatic normals) display a higher ratio of 17 estradiol:estrone. In the hyperlipemic individuals, 94 per cent (50 cases) of those studied exhibited a higher ratio of estrone:17 estradiol.

Studies conducted in our laboratory indicate that a definite relationship exists between the concentration of the serum lipids and that of the more potent estrogens in the blood of normal individuals and hyperlipemic patients.
Factors Influencing the Lipolytic Activities of Post-Heparin Plasma of Hyperlipemic and Hypolipemic Patients (P)


The lipolytic activities of post-heparin plasma in hyperlipemic and in malabsorptive patients have been given varying interpretations by others. Standardization of testing conditions should improve the usefulness of the assay. The post-heparin plasma lipolytic activities (PPLA) of 10 normals, 13 patients with “mixed” hyperglyceridemia, 1 girl with “fat-induced” hyperlipemia, 5 members of a family with hypobetalipoproteinemia and a girl with abetalipoproteinemia were studied by the method of Fredrickson et al. The PPLA of the “mixed-type” hyperglyceridemics ranged from 0.38 to 0.64 μEq.FFA/min./ml., and were similar to those of the normal controls (0.34 to 0.53 unit). The PPLA of the girl with fat-induced hyperlipemia was depressed to 0.09. Two overweight hyperglyceridemic patients were admitted to the hospital for dietary control and PPLA studies. A low-caloric rice diet decreased their PPLA to 0.22 to 0.27 in 2 weeks. Their PPLA remained suppressed on low-calorie 50 per cent fat diet (corn oil: medium-chain triglyceride 1:1). A 900-calorie balanced diet caused further reduction of their PPLA to 0.16 to 0.22. A 40 Gm. fat high-caloric diet raised their PPLA to 0.33 to 0.36 in 2 weeks. Additional alcohol calories had no significant effect on their PPLA, but excessive drinking with decreased food intake lowered their PPLA to 0.14 to 0.23. The PPLA of a girl with abetalipoproteinemia and a man with hypobetalipoproteinemia (both having steatorrhea) were found to be depressed, 0.09 and 0.13 respectively. With improvement of steatorrhea, the PPLA of the hypobetalipoproteinemia adult has ranged from 0.17 to 0.29 units over 18 months. The PPLA of his mother, 2 sisters and a brother with acanthocytosis but no steatorrhea were normal (0.31 to 0.41). Fasting for 18 or more hours depressed the PPLA to below 0.30 units in 4 subjects studied. These data indicate that factors including diarrhea, prolonged fasting, dietary pattern, and calorie intake may influence the PPLA of hyperlipemic and hypolipemic patients.

Mechanisms of Incorporation of Labeled Serum Lipid Fractions by Intimal Cells from Human Atheromatous Plaques (P)

Abel Lazzarini-Robertson, Jr., Cleveland, Ohio

In order to determine mechanisms of incorporation of protein-bound cholesterol-7-αH³ and ¹³¹I and H³-labeled homologous serum low-density lipoproteins into subcellular fractions of human intimal cells, suspension cultures in chemically defined nutrients under known gas phase were obtained by cloning from organ cultures of segments of atherosclerotic (2+ to 3+) human arteries. Uptake rates were followed after sonication in 0.25 to 0.44 M sucrose, differential gradient ultracentrifugation and determination of specific activities by gas flow, liquid scintillation spectrometry, silicic acid thin-layer chromatography and autoradiography.

The results obtained in 82 experiments indicated that cholesterol and/or the lipid moiety of serum lipoproteins were incorporated by intimal cells at a faster rate (78.6 to 92.4 per cent) and preferentially to the protein fraction, without detectable increases in total cell nitrogen content. Furthermore, these uptake rates were found to be ATP-dependent and influenced by hypoxia and total intracellular lipid content.

It is concluded that human intimal cells are able to affect in vitro the physicochemical characteristics of the lipid-binding sites of lipoprotein complexes resulting in secondary release of lipid into the cell and accumulation of protein on the cell surface.

Conditioning Factors Affecting Coagulation Response to Dietary Fat in Man

Kyung Taik Lee, Dong Nuck Kim, and Wilbur A. Thomas, Albany, N. Y.

Conflicting reports regarding the effect of single fatty meals on coagulation in man have appeared. The differing results suggested to us that response may be conditioned by factors such as age and previous diet. We have assessed effects of single fatty meals on several coagulation factors (fibrinogen, prothrombin, thromboplastin activity, thrombelastograph lytic times) and blood lipids in 41 white males divided into 4 groups, with age, percent fat in diet, and serum cholesterol mg. percent respectively given in parentheses: I, medical students (25, 40, 165); II, nondiabetics on low-fat diet (20 months) (63, 18, 184); III, diabetics on low-fat diet (20 months) (67, 18, 194); IV, nondiabetics on regular diet (68, 40, 218).

A high-fat test meal (135 Gm. butter fat) was given after fasting, and blood drawn before, and 3 and 4 hours after the test meal. Marked increases were observed only in a modified thromboplastin generation test (groups I, III & IV), and prothrombin and triglycerides (all groups). When group I was further tested with a corn oil meal (135 ml.), no changes in thromboplastin activity were observed.
The data suggest: (1) many coagulation factors are not altered by long-term low-fat diets or by massive fatty meals; (2) altered thromboplastin generation is produced in some and not in others by single fatty meals, and the response is probably conditioned by multiple factors including age, type of fat, previous diet and diabetes. More needs to be known about the conditioning factors and the relation of these in vitro changes to thrombosis.

Unusual Serum Lipoprotein-Globulin Complex in Hyperlipemia: An Eleven-Year Study

Lena A. Lewis and Irene H. Page, Cleveland, Ohio

There is evidence that the stability of the plasma lipoproteins might have some effect on their atherogenicity. We describe a patient who for at least 11 years has had severe hyperlipemia (cholesterol 800 to 1,100 mg., triglycerides 1,000 to 2,000 mg.). Recent studies of his renal and coronary vasculature show no atherosclerosis. It is suggested that this is due to the stabilizing effect of complexes formed between the lipoproteins and an abnormal globulin.

The serum proteins differed from normal in (1) having high concentrations of abnormal globulin with electrophoretic mobility as measured by free moving boundary or paper technics between the \( \alpha_2 \) and \( \beta \)-globulin—measured on starch gel, it migrated between slow \( \alpha_2 \)-globulin and transferrin; (2) having the abnormal globulin present in lipoprotein concentrates separated by ultracentrifugation, which was identifiable by starch gel electrophoresis; (3) in having 18 per cent of atypical macroglobulin with sedimentation constant \( S_{12} \).

Since Waldenström et al. have some evidence of complexing between abnormal globulins and other serum proteins, we suggest that a complex of abnormal globulin and lipoproteins is present, which might add stability, especially to the \( \beta \)-lipoproteins, and so hinder their precipitation in walls of blood vessels. Neither diet nor a variety of cholesterol-reducing drugs has proven effective in lowering the hyperlipemia.

Effect of Blood Loss on Blood Viscosity in Patients with Chronic Arterial Insufficiency

George A. Mayer, Kingston, Ontario, Canada

Clinical observations have suggested in the past that lowering of the abnormally high blood viscosity may improve the symptoms of chronic arterial insufficiency. Consequently, we studied the effect of venesections on the clinical status and on the absolute viscosity of whole blood and of plasma in 30 patients. Of this group, 25 had chronic coronary heart disease (17 with intractable angina), 2 had intermittent claudication, and 3 basilar or carotid artery insufficiency. All were ambulatory outpatients, and all but 3 were taking anticoagulant drugs on a long-term basis. We removed from 50 to 400 ml. of blood at a time, in 2 to 8 weekly intervals during a period ranging from 6 to 21 months. Microhematocrit and whole-blood viscosity decreased gradually after several venesections. Plasma viscosity decreased in 14 patients, remained unchanged in 12, and increased in 4. Intractable angina markedly improved or subsided in 13 patients, remained unchanged in 2, and became worse in 2, 1 of whom died. Slight exertional angina developed in 3 patients who were free of symptoms before venesections and subsided 4 weeks after the last blood letting. Three patients with basilar or carotid artery insufficiency improved markedly. Two cases with intermittent claudication showed slight improvement. One patient suffering from chronic coronary heart disease and not receiving anticoagulant therapy had weekly blood lettings of 100 ml. over a period of 6 weeks. He died suddenly 5 days after the last venesection.

Effect of Blood Loss on the Beta-Lipoprotein and Lipalbumin Pattern of Serum

George A. Mayer, Kingston, Ontario, Canada

During routine determinations of beta-lipoprotein and lipalbumin by paper electrophoresis in sera of patients, we noted a rise of lipalbumin and a decrease of beta-lipoprotein fractions after blood loss. In a group of 25 subjects (5 healthy, 18 with chronic coronary heart disease, and 2 with other chronic ailments), we withdrew 60 to 150 ml. of blood every 8 to 15 days during a period of 1 to 4 months.

The average individual blood loss was 250 ml. per month, and the average duration of observation 14 weeks. We observed a moderate rise of lipalbumin and \( \alpha_2 \)-lipoprotein, and a significant decrease of beta-lipoprotein fractions after blood withdrawal whenever hematocrit values decreased. After discontinuation of blood lettings, hematocrit increased, but the lipoprotein pattern did not change immediately toward the pretreatment values.

Naturally Occurring Atherosclerosis in the Squirrel Monkey (Saimiri sciurea) (P)

Charles C. Middleton, Thomas B. Clarkson, Hugh B. Lofland, and Robert W. Prichard, Winston-Salem, N. C.

A small, omnivorous, relatively inexpensive sub-
human primate which develops atherosclerosis on natural diets is needed. The squirrel monkey appears to be such an animal. In a preliminary series of 15 animals, a high incidence of atherosclerosis was noted. For confirmation, 15 mature squirrel monkeys were obtained from the same source. After measurement of blood pressure and electrocardiogram, blood was obtained for serum lipid studies and the animals were necropsied. Aortas and hearts were examined grossly and microscopically. Serum cholesterol values ranged from 110 to 235 mg. per cent (mean = 170), and serum triglyceride levels averaged 85 mg. per cent. Ten of 15 animals had gross aortic lesions principally at the arch and near the iliac bifurcation. All animals had microscopic arteriosclerotic lesions. In 10, these lesions consisted of intra- and extracellular accumulations of Sudan-IV positive material, associated with varying numbers of spindle cells. Usually these cells were in greatest number between the lumen and the main collections of lipid. Small granules of basophilic material, often iron positive, were in the lesions and in the adjacent media in some instances. The remaining aortas had groups of spindle cells forming small plaques, without stainable lipid.

Three of 15 animals had atherosclerotic lesions in the coronary arteries. Four of 15 had coronary artery medial fat.

Since the squirrel monkey develops atherosclerosis without dietary manipulation, it is concluded that this animal offers great promise as an experimental animal model for the study of this disease.

**Serum Cholesterol Levels During Long-Term Anticoagulant Therapy in Patients with Complications of Atherosclerosis: Comparison of Effect of Coumarin Derivatives and Phenindione Administered Consecutively**

*John H. Morledge and William B. Parsons, Jr., Madison, Wis.*

Increases in serum cholesterol levels during therapy with coumarin derivatives but not with phenindione have been reported by 2 groups (Herbert et al., Wells et al.). We studied levels of serum cholesterol and lipoprotein cholesterol fractions (beta, alpha,) at intervals of 2 to 4 weeks in 15 patients receiving coumarin derivatives (warfarin or bishydroxycausein) and phenindione in consecutive periods of 3 months' duration. In 28 patients a control period was followed by phenindione administration for 3 months; 15 then received warfarin and 6 received bishydroxycausein for 3 months, while the remaining 7 continued phenindione. Another 23 patients already receiving coumarin drugs were studied for 3 months of coumarin and 3 months of phenindione therapy. Three patients received both coumarin drugs in different periods.

Comparison of final and mean values for the above-mentioned cholesterol determinations showed no significant differences for phenindione, coumarin, and control periods. No correlation between individual changes in cholesterol levels and dosage was observed. Comparison of optimum doses revealed a phenindione/warfarin ratio of 14.9 (mean weekly doses 606 mg./41.8 mg.) in 36 patients, and a phenindione/bishydroxycausein ratio of 1.6 (mean weekly doses 626 mg./431 mg.) in 18 patients. Despite previous inexperience with phenindione and familiarity with the coumarin derivatives, the authors concluded that phenindione provided greater stability of dosage and a greater safety margin (fewer hemorrhagic episodes) while attempting to maintain prothrombin concentration at 10 to 30 per cent activity. Leukocyte counts showed no abnormality except 1 instance of granulocytopenia, probably unrelated to phenindione administration.

**Effect of Fatty Acids on Blood Coagulation, Platelet Economy, and Thrombosis**

*Edmond A. Murphy, Baltimore, Md., Harry C. Bousell, Guelph, Ontario, Canada, Brigitta Hegardt, Toronto, Canada, Harry G. Downie, Guelph, Ontario, Canada, and J. Fraser Mustard, Toronto, Canada*

Because of the relationship of fats to atherosclerosis and the effects, previously demonstrated, of dietary fat on platelets, coagulation and thrombosis, we have explored the effect of saturated and polyunsaturated fats in man and pigs.

Six male subjects in a metabolic ward were fed diets with one-third of their calories as fat, half of this being either ethyl linoleate or ethyl stearate. Dairy fats and egg yolk were eliminated from the diets. Each subject was studied during and for 3 weeks prior to the platelet survival and coagulation studies. No clear-cut differences were encountered in any of the coagulation tests, but in 5 of the 6 subjects, platelet survival was unexpectedly longer during the period when the saturated fatty acid was fed. We did confirm, however, that serum cholesterol was significantly lower when the subjects were fed the unsaturated fat diet.

The effect of fatty acids on thrombus formation was studied by means of the extracorporeal shunt method in swine. Three groups of 10 pigs each received a low-fat diet, a diet containing soybean tristearin, and a diet containing safflower oil, respectively, for a period of 4 weeks. At the end
of this period, coagulation tests and studies of thrombus formation were carried out. Little difference in tests of coagulation and thrombus formation was demonstrated among the 3 groups.

These findings suggest that, whatever importance the dietary intake of these fats has in atherosclerosis, it has little influence on platelet economy, coagulation, and thrombosis.

**Epinephrine, Blood Coagulation, and Thrombosis (P)**

*J. Fraser Mustard,* Toronto, Canada; *Harry G. Downie,* Guelph, Ontario, Canada; *Brigitta Heggard,* Toronto, Canada; *Harry C. Rowell,* Guelph, Ontario, Canada; and *Edmond A. Murphy,* Baltimore, Md.

Studies were done of the effects of intravenous epinephrine at various dose levels on various blood coagulation tests and thrombus formation in 53 swine.

The whole-blood clotting time in glass and silicone was accelerated at doses of 1.6, 3.3, and 33 μg./Kg. body weight. The activity of factor VIII and factor IX was enhanced in the usual assay systems by these doses. These effects were reversed partially or completely by epinephrine doses of 150 μg./Kg. body weight. The platelet count, however, showed a steady increase with dosage throughout this range.

Studies were also done using the extracorporeal shunt method for quantitating thrombus formation in a high-flow pulsatile system. These showed results similar to those for the in vitro tests of blood coagulation. Optimum doses of epinephrine produced a 6-fold increase in the amount of thrombus. Regression analysis showed that there is a significant quadratic component demonstrating that the effect of epinephrine is biphasic. This was true as well for the changes in the clotting tests, except for the platelet count. These findings suggest the effects of epinephrine, and therefore possibly of stress, on blood coagulation are reflected in a tendency to thrombosis. However, studies in animals rendered thrombocytopenic by the administration of P₃₂ have shown that doses of epinephrine which accelerate the clotting times do not enhance thrombosis. Apparently platelets are necessary in a high-flow pulsatile system for the thrombogenic effect of epinephrine to become apparent.

**Free Fatty Acid Concentration as a Determinant of Triglyceride Deposition and Lipoprotein Secretion in the Perfused Rat Liver (P)**

*Paul J. Nestel,* Melbourne, Australia; and *Daniel Steinberg,* Bethesda, Md.

Previous studies in this laboratory have shown that intravenous infusion of norepinephrine into normal dogs can rapidly produce a fatty liver and that repeated subcutaneous doses of epinephrine in oil can elevate serum lipoprotein levels in dogs and in rats. It was proposed that these changes were secondary to the rapid mobilization of free fatty acids (FFA) induced by the catecholamines. In the present studies, rat livers were perfused with red cell-albumin solutions containing low (0.36 μEq./ml) and high (2 to 4 μEq./ml) concentrations of FFA. When the initial FFA concentration was high, the FFA were rapidly removed from the perfusing fluid (28 per cent clearance in a single passage through the liver), and the liver concentration of triglycerides rose as much as 25 to 60 per cent above control values (23 μEq./Gm.) within 90 minutes. The glyceride content of the perfusing fluid showed little or no change when the FFA concentration of the perfusate was low, but increased significantly when it was high. Using palmitate-1-C¹⁴, it was shown that the rate of incorporation into liver glycerides and the rate of appearance in perfusate glycerides increased about 20-fold when the initial FFA concentration of the perfusing fluid was 2 μEq./ml compared with the values obtained at an initial FFA concentration of 0.36 μEq./ml. These studies support the hypothesis previously presented that elevated plasma concentrations of FFA can play a direct role in leading to triglyceride accumulation in the liver and increased output of lipoproteins from the liver into the serum compartment.

**Magnesium of Serum in Arteriosclerosis**

*Alberto Oliveira and Custodio Martins,* Rio de Janeiro, Brazil

Magnesium in serum was determined in 700 healthy men and 300 women between ages 45 and 60. All were tested by x-ray, electrocardiogram and for total lipids and phospholipids in serum and lipoprotein fractions by electrophoresis. Average result was 2.17 mg. per cent (maximum 2.41, minimum 1.93). The same tests were applied to 300 arteriosclerosis patients. Results: higher magnesium concentration, 282 (94 per cent); quantitative alteration principally in beta-lipoprotein, 268 (89.33 per cent); increase of total lipids and phospholipids, 256 (85.33 per cent); no alteration in any test, 6 (2 per cent). More specific results of the 282 with higher magnesium concentration: increase of magnesium only, 18 (6.38 per cent); magnesium and beta-lipoprotein, 14 (4.96 per cent); magnesium, lipids, and phospholipids, 6 (2.13 per cent); magnesium, beta-lipoprotein, lipids, and phospholipids, 244 (86.55 per cent). The 300 arteriosclerosis patients were divided into
Comparison of the Effects of Nicotinic Acid and Aluminum Nicotinate in Hypercholesteremic Patients

William B. Parsons, Jr., Madison, Wis.

In 60 patients receiving nicotinic acid (NAc) and 21 patients receiving aluminum nicotinate (AIN) throughout their first 32 weeks of therapy, serum total cholesterol (TC) levels were tabulated, with similar comparison of beta-lipoprotein cholesterol (BLC) levels in 33 of the NAc patients and all 21 of the AIN patients. Initial dosage was 1.0 Gm. 3 times daily, increased to 4.5 to 6.0 Gm./day after 12 weeks if hypercholesteremia persisted, or decreased to 1.5 to 2.5 Gm./day if nausea occurred. Mean TC levels were reduced promptly (309 to 249 mg. per cent in 2 weeks by NAc, 318 to 255 mg. per cent by AIN), while BLC levels decreased from 222 to 171 mg. per cent with NAc, 239 to 177 mg. per cent with AIN. Mean TC levels after 32 weeks were 222 mg. per cent (NAc) and 237 mg. per cent (AIN), with mean BLC levels of 149 mg. per cent (NAc) and 159 mg. per cent (AIN).

Nicotinic acid caused flushing initially, rarely caused nausea, and infrequently produced elevated serum enzyme levels (SGOT, SGPT, LDH). Aluminum nicotinate minimized flushing, produced more nausea, and caused more serum enzyme elevations, the latter not accompanied by evidence of hepatic damage. Abnormal enzymatic tests, including BSP retention when present, rapidly became normal on discontinuing medication or substituting NAc in equal dosage. Doses higher than 3.0 Gm./day, required in about half of patients receiving NAc, were rarely needed with AIN.

Accuracy of Reports of Body Weight

Lynn S. Perry, Reulah A. Learnard, and Morton D. Schweitzer, New York, N. Y.

Body build is of interest in many epidemiologic investigations of morbidity and mortality in which actual measurements are too costly or difficult to obtain. In connection with a current study of coronary artery disease, a household survey of the Washington Heights area in New York City was conducted to determine how accurately body weight is reported. The relationship of skinfolds, another parameter of body build to weight, was also explored.

A random sample of 400 households was screened for those with at least 2 acceptable adult subjects. The subjects were interviewed individually. Each was asked his own age, height, and weight, and to report the age, height, and weight of the second subject in the household. After questioning, both were weighed and measured for height and skinfold thickness (subcapular and triceps).

The correlation between self-reported weight and scale weight was greater than r = 0.90 for the total sample. In general, reports were more accurate from women than men, and from people under 45 years of age as compared to those over 45. Findings were similar for comparisons of "second-hand" reports of weight and actual scale readings. The correlation between the selected skinfold measurements and per cent weight deviation (calculated from a standard of average weights) was better than r = 0.70, indicating that skinfolds may be useful as an added dimension.
for describing body build. It may be concluded that reported weight, including that obtained from a second household source, can be substituted for actual scale weight in the population studied.

Environmental Influences on Diet-induced Atherosclerosis in Cockerels

Ruth Pick,† Louis N. Katz, Dolores Century, Philip Johnson, and Chizuko Kakita, Chicago, Ill.

Group-isolation of cockerels has been shown to increase the atherogenic response to a cholesterol-oil diet and decrease the regression rate of lesions when a regular mash diet is substituted for the atherogenic regimen. The present experiments were initiated to study mechanisms responsible for the atherogenesis-potentiating action of group-isolation. Forty cockerels, 10 per tier, were put into a specially constructed, soundproof isolation booth. They were fed and cleaned through slats, without opening the booth. Watering was done by an automatic valve arrangement. Only 2 of the 4 tiers were equipped with wall mirrors. The birds in all 4 tiers could hear all birds in the cage, but only the 2 tiers with the mirrors “saw” birds presumably other than those within their own group. A third (control) group was kept in the regular chicken room housing approximately 500 birds. All animals were fed identical diets.

Results: Growth and sexual development was retarded, as previously described, in both isolated groups. Atherogenesis was enhanced only in the isolated group without mirrors, while the isolated group capable of “seeing others” had a similar degree of atherosclerosis as the control group in the regular chicken room. These results suggest that visual isolation is important in the atherosclerosis-potentiating effect of group-isolation.

New Angiographic Technic Providing a Simultaneous Permanent Cast of the Coronary Arteries (P)

Stanley L. Robbins, Sandra Fish, and Felix L. Rodriguez, Boston, Mass.

To date there is no entirely satisfactory method of quantitating or grading coronary artery atherosclerosis. A semirigid plastic has been devised that provides for comparative analysis 3 kinds of material on the individual heart: (1) stereoscopic angiograms; (2) an exact cast of the lumens of the coronary trunks and their major branches; (3) the intima, opened vessels.

The injection medium consists of combined rigid- and flexible-type polyester resins, powdered metal filler (copper or tin), and a nonionic surface-active parting agent. This medium is highly radio-opaque, provides an excellent angiogram when used according to a modified Schlesinger’s angiographic technic, cures in 1 hour to a semirigid cast that provides an exact three-dimensional replica of the coronary lumen and its deformations, and then separates readily, leaving the uninjured opened coronaries for morphologic examination.

The new injection medium permits the correlation of radioangiography and morphologic study of atheromata with the cast of the vessel that constitutes a permanent precise record of the functional lumen of the vessel. In a series of human hearts so studied, data have been collected on the cross-sectional effects of coronary atherosclerosis on coronary patency and hence blood flow.

Intercoronary Arterial Anastomoses in Normal Young Adult Pigs


There are conflicting views on the incidence of large intercoronary arterial anastomoses in normal pig hearts. Some claim that such anastomoses often occur; others that they do not. The importance of this controversy stems from the fact that, in assessment of the efficacy of procedures or drugs for inducing the formation of intercoronary anastomoses, the pig is considered the experimental animal of choice.

The hearts of 55 normal young adult (6 to 8 months) pigs were studied by postmortem coronary arteriography. A part of the coronary arterial tree (one of the major trunks, a large branch, or a second order branch) was injected at 200 mm. Hg for 5 minutes with Schlesinger’s barium sulfate gelatin mass; flow of mass into other parts of the coronary tree was noted (the mass penetrates regularly to arterioles 30 to 40 microns in diameter, but does not enter capillaries). Gross anastomotic routes for such flow were identified on stereoangiograms of the unrolled heart.

In 41 specimens out of 55 (75 per cent), large intercoronary arterial anastomoses at least 40 microns in diameter were observed bridging the main trunks, branches of the same major coronary, or different segments of the same coronary branch. In many hearts, these anastomoses and accompanying veins were observable on the endocardial surface of one or both ventricles.

Review of the literature suggests that the reported conflict on the normal existence of such anastomoses in the pig is probably due to differences in technic.
Arteriosclerosis in Whales and Dolphins


Postmortem studies of the coronary arteries, aortas, and hearts from over 40 cetaceans (whales and dolphins) of various species from both wild and captive (Marineland of the Pacific) environments have been made. Correlations with serum lipids, body fat and diet were also made.

The intimal lesions in the vessels were of all stages of severity. All lesions were remarkably similar, in both gross and microscopic appearance, to lesions of human atherosclerosis.

Effect of Chlorophenoxyisobutyric Ester Alone and in Combination with Androsterone on Serum Lipids


A double-blind evaluation of the serum lipid effects of 3 test substances was carried out: (1) a placebo, (2) chlorophenoxyisobutyric ester, and (3) chlorophenoxyisobutyric ester in combination with 2.5 Gm. androsterone daily by mouth. Forty-five middle-aged male patients with hypercholesterolemia were chosen for the study. Control lipid studies (total cholesterol, lipid phosphorus, triglycerides, α and β lipoproteins, and a light β fraction with a specific gravity of less than 1.006) were made at monthly intervals for at least 2 months. Then, 15 patients were assigned to each of the 3 test substances for 3 months. Lipid determinations were repeated each month. Posttreatment placebo controls were obtained.

At the end of the 6-month study, no significant change had occurred in the lipid values of the 15 controls. In the group receiving chlorophenoxyisobutyric ester, there was a reduction in the mean total cholesterol from 292 to 260 mg. and the β lipoprotein cholesterol from 221 to 175 mg.; the very light β fraction decreased from 20 to 14 mg.; triglycerides were slightly lower, from 111 to 92 mg. The combination of chlorophenoxyisobutyric ester with androsterone produced more significant reduction in total cholesterol (from a mean of 286 to 235 mg.) and in β lipoprotein cholesterol (from 292 to 192 mg.); the light β fraction decreased from 38 to 22 mg. The triglycerides were 144 in the control period and 133 mg. after 3 months of therapy.

Effect of Conjugated Equine Estrogens on the Clotting Mechanism


Conjugated equine estrogens have been reported to produce favorable shifts in serum lipids and to increase the length of survival after a first attack of myocardial infarction. They have also been used empirically to stop bleeding by increasing the strength of the capillary wall. This might lead to clot formation which would be undesirable in the atherosclerotic patient.

To test the effect of conjugated equine estrogens on the clotting mechanism, 27 patients were selected for study. There were 14 women and 13 men. The age range of the patients was from 38 to 72 years. Silicon clotting time, platelet counts, platelet adhesiveness on glass beads, prothrombin time, thromboplastin generation, and prothrombin consumption tests were carried out immediately before and 1½ hours after 20 mg. of conjugated equine estrogens were given intravenously.

The average mean control platelet count, using a phase microscope, was 266,000. This decreased an insignificant amount to 252,000. The platelet adhesiveness on glass beads was 55 per cent before, and again 55 per cent after the intravenous medication. The thromboplastin generation test increased from 59 to 65 seconds (well within the normal range of 40 to 100 seconds). The prothrombin consumption time was unchanged from 44 to 45 seconds.

There was no evidence obtained by these methods to indicate that conjugated equine estrogens increased the clotting tendency 1½ hours after the intravenous injection of 20 mg.

Similar results were obtained in 10 women after oral administration of 1.25 mg. of conjugated equine estrogens for 1 month.

Incidence and Topography of Coronary Oclusions; Relation to Coronary Anatomic Pattern


An angiographic study of 430 nonselected hearts disclosed 3½ times more coronary occlusions, and 2½ times more hearts with occlusions than were found in control routine autopsies.

The 430 hearts were from 372 whites, 56 Negroes, 2 Chinese; 238 men, 192 women. Mean age was 68 (S.D. 14) years; two-thirds were at least 60 years old. All but 11 subjects were Christian. Hearts with occlusions numbered 103; number of occlusions was 227.

The topography of occlusions demonstrated angiographically was similar for either sex. The majority of occlusions were short (0.5 cm. or less). Two-thirds were lodged within 4.0 cm. of the coronary ostia. Coronary main stems were occluded twice more often than branches. The right coronary artery was occluded as often as that of the left anterior descending; that of the left circum-
flex was occluded the least often. Occlusions in the right coronary main stem ranged farther away from the coronary ostium than those in the other main stems. The same sites in the coronary tree were favored by solitary occlusions and multiple ones. Occlusions occurred as often in hearts with (Schlesinger’s) balanced coronary pattern as in those with a right or a left preponderant pattern. Occlusions did not involve the preponderant coronary more often than other coronaries.

The demonstrated incidence and topography of coronary occlusions in this series resemble those reported for another series, largely Jewish, similarly studied by M. J. Schlesinger over 2 decades ago.

Evaluation of Treatment of Patients Sixty-five Years of Age and Over for Hypercholesteremia

Paul B. Roen, Los Angeles, Calif.

Evidence is sought to ascertain whether the treatment for hypercholesteremia is of value in patients 65 years of age and over. Treatment has been principally low-lipid diet with the unsaturated fatty acids and the hormones—thyroid and estrogen—besides some of the current drugs.

There were 169 recorded deaths since 1949 among the 1,627 patients that have been studied at the Clinic and in private practice. Of these, however, there were only 18 (11 males and 7 females), about 11 per cent of the total, who had been faithful to their treatment for periods ranging from 4 to 14 years who died directly from arteriosclerosis.

Based on the 1958 CSO Mortality Tables used by life insurance companies, the life expectancy was added to the patient’s age when treatment was started. It was found the sum total of years of those who did not live their expected age was 40.68 years, and for those who lived longer the sum total was only 15.87 years. Thus it appears for those 65 and over, under treatment for hypercholesteremia, the sum total of those who died before their expectancy was 2.7 times those who lived over their life expectancy. Thus it seems that the treatment had no effect on increasing longevity, rather the opposite. Evidently, if life expectancy is to be increased, the treatment will have to be started earlier in life.

Effect of Thrombocytopenia on Thrombosis and the Endothelium

Harry C. Rousell, Guelph, Ontario, Canada, J. Fraser Mustard, Toronto, Canada, Gerald A. Robinson, Guelph, Ontario, Canada, and Brigitta Hegardt, Toronto, Canada

Although there is considerable indirect evidence that the platelet is a primary factor in the formation of arterial thrombi, there is little direct evidence that it is an essential component. Swine therefore have been rendered thrombocytopenic by the administration of P32 to determine whether thrombi can form in a high-flow pulsatile system when there are no circulating platelets. Thrombus formation was estimated, using the quantitative extracorporeal shunt technic previously described. Ten young pigs with platelet counts less than 10,000 per cmm. formed no thrombi in the extracorporeal shunts. Twelve pigs that had platelet counts ranging between 10,000 to 200,000 per cmm. showed a positive correlation with the amount of thrombus. Epinephrine injections which had been shown to enhance thrombus formation in earlier experiments did not enhance thrombus formation in thrombocytopenic swine. Similar observations were found for injections of adenosine diphosphate (ADP), which in normal pigs enhance thrombus formation.

Endothelial membranes prepared from thrombocytopenic pigs showed evidence of loss of cement lines, and the appearance of what appeared to be gaps between the endothelial cells. These results show that, in a high-flow pulsatile system, the platelet is necessary for thrombus formation and for maintaining endothelial morphology.

Cholesterol Synthesis from C14-Acetate, and I131-Trio- lein Absorption Studies in Patients Treated with Para-aminosalicylates


In all of 14 hospitalized patients, total serum cholesterol was reduced by an average of 29 per cent by daily oral administration of 9 to 12 Gm. of para-aminosalicylic acid of 6 to 19 weeks. Only 7 of the 14 patients, who received 12 Gm. of sodium para-aminosalicylate daily, had significant cholesterol reduction. I131-triolein was given orally to 10 patients in 21 instances, during control periods and after PAS administration. In 6 subjects with significant cholesterol reductions, the average blood radioactivity for the first 8 hours was 9.5 per cent during control periods, and 8.3 per cent during PAS administration. The 72-hour fecal radioactivity was 5.6 per cent in both groups. In 5 patients without significant lowering of serum cholesterol, the blood levels were 10.9 and 11.9 per cent, and the fecal excretion 2.2 and 8.1 per cent respectively during control and PAS periods. These differences were not statistically significant. Three patients following the reduction of serum cholesterol by PAS, received 100 μc. of sodium acetate-l-C14 intravenously.
Duplicate serial serum samples were processed according to Gould et al., and read in a liquid scintillation counter. Total cholesterol peak radioactivity occurred in 2 to 6 hours in each subject, reaching 0.154, 0.106 and 0.061 μg./Gm. cholesterol, with serum cholesterol values of 148, 305 and 266 mg. per cent respectively in the 3 patients. The reduction of serum cholesterol by PAS appears to have no demonstrable influence on the rate of acetate incorporation into endogenous cholesterol.

Comparative Morphology of Aortic Intimal Smooth Muscle and Elastic Tissue in Rabbit and Rat

Eric A. Schenk,‡ Rochester, N. Y.

Recent studies dealing with the pathogenesis of experimental atherosclerosis have focused on the role of intimal smooth muscle in the formation of foam cells. Present concepts as to the aortic intimal structure hold that the endothelium lies directly on an elastic lamina. The question of whether or not smooth muscle is normally present in the intima is the subject of this report. A modified Haueter technic was used to strip successive layers of aortic wall. Cytomorphological analysis of these layers of rabbit aorta showed that the innermost elastic lamina is composed of densely packed elastic fibrils oriented predominantly parallel to the long axis of endothelial cells. These fibrils form a matrix for smooth muscle cells arranged circumferentially and at right angles to the elastic fibrils. The smooth muscle cells lie directly adjacent to endothelium in areas of elastic lamina discontinuity, or fenestrae. The smooth muscle cells contain myofibrils, as well as a component which stains like connective tissue, and may be thought of as modified mesenchymal cells. Elastic lamina fenestrae, and therefore subendothelial smooth muscle, are most prominent in the upper thoracic aorta, in the abdominal aorta, and, in general, along the posterior aspect of the vessel. The rat aorta shows relatively fewer subendothelial smooth muscle cells and elastic lamina fenestrae, and the former are arranged in a spiral to more hazardous manner. Preliminary work suggests that areas of highest smooth muscle concentration show predilection for experimental atheroma formation.

Cholesterol Linoleate Levels of Koreans on High, Intermediate and Low Fat Diets, and of American Soldiers (P)

R. Foster Scott, Kyu Taik Lee, Ethel S. Morrison, and Fairfield Goodale, Albany, N. Y.

Fatty acids of cholesterol ester of several groups of young male Koreans (total 56) and of 16 U. S. Army soldiers stationed in Korea were measured. The groups’ mean serum cholesterol (mg. per cent) and approximate daily cholesterol intake were: I. farmers, 94, 30 ± mg./day; II. monks, 116, 0 mg./day; III. middle-class professionals, 135, 300 ± mg./day; IV. Korean soldiers eating Korean Army diet, 148, 300 ± mg./day; V. Korean soldiers eating American Army diet, 200, 700 ± mg./day; VI. American soldiers in Korea, 200, 700 ± mg./day. The total fat in the diet varied from ± 20 Gm./day (40 per cent polyunsaturates) in monks and farmers to ± 135 Gm./day (10 per cent polyunsaturates) in the American Army diet. Absolute values for cholesterol linoleate in mg. per cent (relative per cent in brackets) were I, 33 (44); II, 38 (47); III, 54 (53); IV, 42 (55); V, 73 (50); VI, 69 (52). Thus total cholesterol levels rose in direct relation to tremendous increases in dietary cholesterol, but cholesterol linoleate rose at an even greater rate, although dietary polyunsaturates increased only slightly in absolute amounts and decreased markedly in relative amounts. This indicates a potent control mechanism which may vary in efficiency since there were marked variations in individual values within groups. This study points up need for specific information on atherogenic effect of various “cholesterols.” For example, cholesterol linoleate could be either more or less atherogenic than other cholesterol, and individual variations in amount of atherosclerosis may be related to metabolic efficiency in producing this component.

Tetracycline-induced Alterations in Serum Lipoproteins (P)


Although the antibiotic effectiveness of tetracyclins is well established, little is known about serum transport of these agents. To study possible protein interaction, small quantities of tetracycline analogues were added to pooled human serum. Such treatment with tetracycline phosphate (TCP) or chlortetracycline (CTC) enhanced serum lactescence, whereas demethylchlortetracycline (DTC) produced frank precipitation. By contrast, oxytetracycline (OTC) did not alter serum appearance. Electrophoretic strips prepared from serum-tetracycline mixtures exhibited distinctive patterns of fluorescence in ultraviolet light. Staining strips with Oil Red O demonstrated that fluorescent zones corresponded to electrophoretic areas rich in lipid. With the exception of OTC, tetracyclines decreased electrophoretic mobilities of serum lipid-bearing
proteins. The entire lipoprotein spectrum of serum CTC or TCP mixtures was confined to a narrow electrophoretic band near the point of application. Such electrophoretic behavior may be ascribed to alterations in charge on the protein or possibly denaturation.

Addition of calcium to serum containing TCP, CTC or DTC produced prompt precipitation. Aside from small diminutions in globulin, supernatants showed electrophoretic protein patterns similar to untreated serum. Oil Red O-treated electrophoretic strips containing supernatants failed to disclose lipophilic material. Hence, lipoprotein precipitation may account for modest declines in globulin content of supernatants. Immunochromatographic analyses indicated that serum lipoproteins of density < 1.063 Gm./ml. were removed by addition of CTC or DTC and calcium. Moreover, such supernatants did not contain detectable amounts of cholesterol. These data suggest that certain tetracyclines are capable of interacting in vitro with serum lipoproteins which are rendered insoluble by calcium.

Incidence of Hyperlipemia and Hypercholesterolemia in an American College Population

_Winnifred Seegers,‡ and Kurt Hirschhorn,† New York, N. Y._

We examined sera of 2,138 college freshmen from New York, Chicago, and Seattle for optical density (O.D.), triglycerides, and cholesterol. Since these were not fasting samples, we tested the relationship between O.D. and triglycerides to differentiate alimentary from true hyperlipemia. The formula, (casual) triglycerides²/O.D., was derived from the relationship between casual and fasting samples from 100 medical students. A value was obtained dividing individuals with fasting triglycerides below 100 mg. per cent and those above 150 mg. per cent, the borderline group being distributed above and below this value.

By using this discriminant (triglycerides²/O.D.), we analyzed the large college population for the incidence of hyperlipemia and hypercholesterolemia, as correlated with sex, geographical, religious and national origins, and family history of heart disease. Despite the casual nature of the samples, serum triglycerides were under 100 mg. per cent in 531 (88 per cent) of 605 females and 302 (58 per cent) of 521 males from 3 of the schools. Since within any O.D. range, females have lower triglycerides than males, this difference is not determined only by the amount eaten, but may be due to protective factors in the female internal environment. This is reflected by a sex difference in the incidence of hyperlipemia. Of individuals with triglycerides over 100 mg. per cent, 13 of 74 females (2 per cent of all females) and 53 of 219 males (10 per cent of all males) were classified as hyperlipemias by our criteria.

Coronary and Aortic Arteriosclerosis in Free-ranging and Captive Woodchucks (Marmota monax)

_Robert L. Snyder and Herbert L. Ratcliffe, Philadelphia, Pa._

Aortas of 907 and coronary arteries of 326 woodchucks (Marmota monax) were examined to determine the nature and prevalence of aortic and coronary arteriosclerosis in a free-ranging population. Young animals from this population aged alone or as heterosexual and homosexual pairs in the laboratory contributed additional material.

Medial degeneration with calcification (Mönckeberg’s sclerosis) was found in 6 aortas. Lesions of the coronary arteries were more numerous. These consisted of intimal thickening with duplication, fraying or disappearance of the internal elastic. Degeneration or hyalinization of media often accompanied the intimal change in larger arteries. The disease most often involved the intramural arteries and the arterioles and increased in frequency and severity with age. Minimal lesions, usually involving only small intramural arteries and arterioles, were found in about half of woodchucks less than one year old. Advanced lesions, with distinct narrowing of lumens and involvement of major coronaries, were found in 3-year-old woodchucks.

Six of 24 animals in the laboratory died of cardiovascular-renal disease within 2 years of age. Causes of death were ruptured aorta (2), infarction of myocardium (1), and glomerulonephritis (3). Dissection and rupture of the aorta were associated with medial necrosis. Coronary arteries of all 6 animals showed evidence of necrosis and hyalinization often accompanied by perivascular hemorrhage through damaged arterial walls. Since each of these woodchucks also had severe renal disease, we suggest that altered renal physiology may have been a major factor in the acute necrotizing process that was responsible for the arterial damage.

Prevalence of Diabetes Mellitus and Correlated Abnormalities in Low-Income Chicago Negroes

_Jeremiah Stamler, Rose Stamler, David M. Berks, Monte Levinson, Louis Kolokoff, and Samuel I. Andelman, Chicago, Ill._

During 1962, a special multiphasic chronic dis-
ease detection survey was accomplished among the population age 30 and over of a Chicago public housing project whose residents are predominantly Negro. One significant finding, revealed by glucose tolerance testing, was a high prevalence rate of probable diabetes mellitus—177 per 1,000 overall. The overall rates were 160 and 181 per 1,000 in men and women respectively; in the age group 30 to 49, they were 104 for men and 73 for women. The diabetes rate in the obese was approximately twice as high as in persons at or below desirable weight. Prevalence rates of hypercholesterolemia and hyperuricemia were significantly higher in the probable diabetics, compared with the nondiabetics. Prevalence rates of hypertension, abnormal electrocardiograms, and total cardiovascular abnormalities were 2 to 3 times greater in the probable diabetes, compared with the nondiabetes. These findings indicate that Negroes have higher rates of diabetes than hitherto reported in other Americans, and that observed multiple abnormalities may play a significant role in accounting for the inordinately high rates of cardiovascular disease in middle-aged U. S. Negroes.

Relative Importance of Dietary Cholesterol and Unsaturated Fats on Serum Cholesterol Level of Man

Alfred Steiner, Elliott Howard, and Anna Wilson, New York, N.Y.

Two studies have been made to evaluate the relative importance of dietary cholesterol and unsaturated fats on the serum cholesterol level of man.

1. A diet, composed of naturally occurring foods high in fat (100 to 290 Gm., 80 per cent unsaturated), moderate in protein (70 to 160 Gm.) and low in carbohydrate (10 to 20 Gm.), containing 1.3 to 3 Gm. of cholesterol, was fed to 5 patients for 6 weeks. The serum cholesterol level remained unchanged in 3, and increased significantly in 2 of the patients.

2. The formula diet technic was utilized to administer a diet (C-280, P-70, F-90), the fat content of which consisted entirely of corn oil, with either 3 Gm. or 1.5 Gm. of added cholesterol. At the 3 Gm. cholesterol intake, the serum cholesterol level increased, while at the 1.5 Gm. cholesterol addition, the serum cholesterol level decreased. The decrease in the serum cholesterol level on this latter diet was abolished when one-quarter of the corn oil was replaced with coconut oil.

The results of this investigation suggest that the ratio of the cholesterol to unsaturated fat content of the diet may play an important role in the elevation or reduction of the serum cholesterol level of man.

Ophthalmoscopic Evaluation of Retinal Arteriosclerosis and Serum Cholesterol


It is generally agreed that increased and interrupted light reflex, uneven caliber, and arteriovenous crossing changes, all of which may or may not be accompanied by an increased tortuosity, are indications of retinal arteriosclerosis as seen by retinoscopy. The possible relationship between the highest levels of serum cholesterol and serum lipids and the incidence of these funduscopic abnormalities was examined.

A group of 325 active Chicago businessmen who have been undergoing detailed annual physical examinations are the subjects of the study. Their ages varied between 28 and 72. Sixty-five per cent were between ages 40 and 60. Cholesterol, total lipids, and phospholipids were determined on fasting sera.

One hundred twenty-four individuals had evidence of retinal arteriosclerosis, whereas 48 had blood pressures in excess of 150/90. The serum cholesterol level was above 250 mg./100 ml. in 55 per cent of the group, and under 225 mg./100 ml. in 24 per cent. Employing either of these values as the upper limit of normal for a X² test, no difference in incidence of abnormal arterial findings could be found between the groups above or below this level. The same lack of relationship was also present in the group under 40 years of age. Retinal arteriosclerosis, serum cholesterol, and the Halstead impairment index, used as a parameter for higher brain functions, all correlated with age.

Nephrosis: Relationship of Hypoalbuminemia to Hyperlipemia

Richard E. Tracy, Chicago, Ill.

Allen, Baxter, and Goodman have reported that bovine serum albumin (BSA) injected into nephrotic rats in massive doses raises the serum albumin concentration and lowers the serum lipid level. This evidence has been used to support the widely held hypothesis that hyperlipemia in nephrosis may be caused by hypoalbuminemia. The results of the present study do not support this hypothesis.

After nephrosis was induced in rats with anti-kidney serum, BSA was given intraperitoneally for 2 days at a dosage lower than that reported by the above authors (4 Gm./Kg./day), and tube feeding was used to control the diet carefully.

The results presented in the table indicate that under these experimental conditions, a substantial
elevation of serum albumin was not accompanied by a significant fall in serum lipid level.

<table>
<thead>
<tr>
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<th>Serum albumin</th>
<th>Serum total lipid</th>
<th>Albuminuria mg.%/12 hr.</th>
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<tr>
<td>Normal rats</td>
<td>2.60</td>
<td>3,424</td>
<td>15</td>
</tr>
<tr>
<td>Nephrotic controls</td>
<td>2.05</td>
<td>1,978</td>
<td>159</td>
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<tr>
<td>BSA injected</td>
<td>4.52</td>
<td>1,045</td>
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Fatty Acid Composition of Normal Rabbit Plasma and Its Fractions


The lack of detailed information on the fatty acid composition of normal rabbit plasma and its lipid fractions, in spite of the wide use of this animal in atherosclerosis research, has led to the following study.

Plasma from normal rabbits was extracted and the various lipids were separated by means of silicic acid and florisil column chromatography. The purity of the fractions was tested by microchemistry and thin-layer chromatography, following which detailed analysis of the fatty acid composition of each individual fraction was undertaken by means of gas-liquid chromatography. A series of fatty acids from C-8 to C-20 with varying degrees of unsaturation were found to be present. The most prominent were: 16:0 (27 per cent), 16:1 (3 per cent), 18:0 (9 per cent), 18:1 (16 per cent) and 18:2 (31 per cent). Four per cent of the fatty acid was in the form of C-20 with up to 5 double bonds.

Interesting variations in the fatty acid composition were found between cholesterol esters (C.E.), phospholipids (P.L.), and triglycerides (T.G.). Fatty acid 16:0 comprised 16 per cent of C.E. and P.L., while the T.G. was 25 per cent. The stearic acid content was 5 per cent in C.E., 7 per cent in T.G., and 14 per cent in the P.L. fraction. C-18:2 showed 47 per cent in C.E., 29 per cent in T.G., and 52 per cent in P.L. The presence of 20:3 was negligible in C.E. and T.G. and comprised 4 per cent of the P.L. fatty acids; 20:4 was relatively high in the C.E. fraction (2 per cent) and negligible in the T.G. and P.L. fractions.

Meniscography: A New Method in Coagulation and Fibrinolytic Studies

P. Udden, Kälarne, Sweden

In order to overcome the difficulties in recording the prothrombin time, especially difficult at "close-to-bleeding" levels, a new method of recording blood coagulation has been developed. The method is based on a pumping system (0.5 to 20 mm³) which works over an air column on a sample (10 to 200 mm³) in a plastic tube (diameter 0.5 to 2 mm.).

The sample volume, the tube diameter, the pumping volume, and pumping frequency can be so chosen that either the viscosity or surface tension is measured. In coagulation and fibrinolytic studies, "meniscography" has been shown to be the best to work with. As the recording is made in exchangeable plastic tubes, there are no cleaning troubles as, for instance, in thromboelastography. With this method, coagulation studies can be performed also in Teflon tubes. By use of thrombocyte-free plasma and specially prepared lipid factor (thrombocyte factor 3), standardized conditions can be achieved for coagulation-time recording.

Smooth Muscle Cell Necrosis as a Significant Factor in the Pathogenesis of the Vascular Changes in Arteriolar Nephrosclerosis


The essential vascular lesion of arterial hypertension is hyaline arteriolar sclerosis. It is especially marked in the kidneys, where progressive arteriolar narrowing leads to ischemic atrophy (arteriolar nephrosclerosis). In an investigation of the pathogenesis of this lesion, the fine structure and the histochemistry of citric acid cycle and high-energy hydrolytic enzymes have been studied in renal arterioles obtained postmortem and by biopsy from patients with "benign" hypertension. The areas of arteriolar hyalinization are devoid of enzyme activity, indicating a lack of viable functioning cells. Lipid is often present as fine droplets. Fine structure studies with the electron microscope show that granular amorphous material forms the hyaline which lies in subendothelial, intimal, and muscular layers. In some hyaline deposits, remnants of cell membrane systems and intracellular organelles, including mitochondria, are present. These are remnants of necrotic smooth muscle cells. Apparently these intracellular membranes, phospholipid-protein complexes, resist autolytic enzyme removal and persist in situ in the arteriolar wall. It seems likely that physiologically perfusing plasma proteins are trapped in this cellular debris, fusing with partially disintegrated cells and ground substance to form the hyaline deposits. These findings support the idea that a cellular lesion may be of prime importance in the pathogenesis of arteriolar sclerosis. Presumably such muscle cell degeneration and necrosis may be initiated or accentuated by the severe and prolonged arteriolar vasoconstriction characteristic of hypertension.
Atherogenic Indices: A Review and Evaluation

Moses Wurm, Reuben Straus, and Robert J. Kositchek, Burbank, Calif.

A considerable number of variables have been studied in an attempt to establish a reliable index for the assessment of the atherosclerotic status in man. While significant differences can be demonstrated between normal and abnormal populations, there has been a singular lack of success when these tests are employed on an individual basis. Current attitudes regarding usefulness of certain biological parameters proposed as atherogenic indices have been colored with emotional preference or used because of the lack of alternative simpler procedures. To assess each of the proposed atherogenic indices we have employed a simple arithmetic procedure, based upon a partition of a mixed population in a 4-fold contingency table. By this technique, the number of individuals successfully associating normal value ($K_1$) with the normal population ($P_n$), and similarly the number of individuals successfully associating abnormal value ($K_2$) with the abnormal population ($P_a$), are determined, and the per cent of correct associations for the entire population is mathematically calculated as the confidence ratio. Indices with confidence ratios greater than 0.75 are considered satisfactory, provided elements of bias are excluded between contrasting populations. By means of this technique, only 6 indices were considered satisfactory by virtue of high confidence ratios and absence of elements of criticism. These are the $^{131}$I-labeled triolein, test meals, ballistocardiography, per cent alpha-cholesterol by cold ethanol fractionation, $S_2$ 10-20 lipoproteins by lipocentrifugation, and the beta/alpha lipoprotein ratios by zone electrophoresis. Blood cholesterol was found not to be a reliable index of the atherosclerotic state.

Fatty Acid Composition of Plaque and Tissue Lipids from Pigeons with Spontaneous Atherosclerosis

Franklin Young, Charles C. Middleton, Hugh B. Lofland, Harold O. Goodman, and Thomas B. Clarkson, Winston-Salem, N. C.

Sixty White Carneau pigeons (5 to 8 years) with spontaneous atherosclerosis were divided into 6 groups. The tissues collected and pooled according to groups were: excised atherosclerotic plaques, aorta proximal to the diseased area, liver, and serum. Lipids extracted from all tissues were separated into 3 major fractions: cholesterol ester (I), triglyceride (II), and phospholipid (III). Methyl esters of the component fatty acids from each fraction were analyzed by gas liquid chromatography. Descriptive statistics (means, standard deviations) and simple and partial correlation coefficients were obtained using a 1620 computer.

The relative percentages of the major fatty acids in various lipid fractions of plaque, nondiseased aorta, liver, and serum are respectively as follows: (I) 44, 24, 44, and 32 for oleic, 31, 19, 14, and 50 for linoleic, 13, 19, 22, and 10 for palmitic; (II) 44, 42, 42, and 46 for oleic, 24, 24, 21, and 21 for palmitic, 12, 11, 17, and 18 for linoleic; (III) 32, 22, 14, and 16 for palmitic, 26, 27, 35, and 34 for stearic, 21, 23, 15, and 17 for oleic, 9, 9, 26, and 26 for linoleic.

The data suggest major differences in the fatty acid composition of (I) from the 4 tissues. The fatty acid composition of (III) from plaque is similar to that of nondiseased aorta. Likewise, the fatty acid composition of (III) from liver resembles that of serum. However, the fatty acid compositions of (III) from plaque and nondiseased aorta were quite different from those of liver and serum.