ASSOCIATION PRESENTS AWARDS AT SCIENTIFIC SESSIONS

Among the honors conferred on physicians for outstanding service in advancing the heart program and for achievement in cardiovascular research by the American Heart Association at its Scientific Sessions, were the following:

**Gold Heart Awards**

The Gold Heart Awards were presented at the Annual Dinner to Edgar V. Allen, M.D., past-President of the American Heart Association and Senior Consultant in Medicine at the Mayo Clinic; David D. Rutstein, M.D., Professor of Preventive Medicine, Harvard Medical School; and Samuel A. Levine, M.D., Clinical Professor of Medicine, Harvard Medical School.

Dr. Allen and Dr. Rutstein were cited for their contributions in advancing the objectives and program of the Association. Dr. Levine was honored for his outstanding achievements in clinical cardiology.

**Lasker Award**

Robert E. Gross, M.D., Professor of Children's Surgery, Harvard Medical School, received the Albert Lasker Award of the American Heart Association for distinguished achievement in the field of cardiovascular research and especially for his pioneering experimental work in the field of cardiovascular surgery. The award consists of a statuette of the *Winged Victory of Samothrace* and an honorarium of $2,500.

FILMS ON STROKE MANAGEMENT ISSUED BY ASSOCIATION

A professional film on strokes of interest to the general practitioner has been issued by the American Heart Association. The film, "Cerebral Vascular Diseases—The Challenge of Management," shows technics for recovery and rehabilitation through use of services and equipment available to every physician with a stroke patient. It stresses the need for early and continued care and for cooperation of the patient and his family throughout the recovery period.

Many affiliates and chapters of the Association are planning showings of the film to physician audiences in connection with scientific programs on the problem of rehabilitating stroke patients.

"Second Chance," a lay film based on the same case history, has also been produced for the Association and its affiliates. It can be of help to physicians in instructing families of victims on rehabilitation procedures and as a visual aid in addressing non-professional groups.

Both are 16mm. black-and-white films, produced and directed by George C. Stoney and Associates for the American Heart Association. "Second Chance" is available to physicians on loan from local Heart Associations or from the American Heart Association, 44 East 23rd Street, New York 10, N. Y.

A second professional film on strokes,"Cerebral Vascular Diseases—The Challenge of
Diagnosis,” is in preparation for early release by the Heart Association.

MEETINGS CALENDAR


November 12-13: International Symposium on Cardiology in Aviation, Texas. Lawrence E. Lamb, Department of Internal Medicine, School of Aviation Medicine, USAF, Brooks Air Force Base, Texas.

November 13-14: Annual Symposium on Cinefluorography, Rochester. George H. Ramsey, Department of Radiology, Strong Memorial Hospital, Rochester 20, N. Y.


1960


March 26-27: American Psychosomatic Society, Montreal. Eric Wittkower, 265 Nassau Road, Roosevelt, N. Y.

March 28-31: Southwestern Surgical Congress, Las Vegas. Mary O'Leary, 1213 Medical Arts Building, Oklahoma City, Okla.

April 1-3: American Society of Internal Medicine, San Francisco. R. L. Richards, 350 Post Street, San Francisco 8, Calif.


ABROAD

1960

May 2-11: Pan American Medical Association Congress, Mexico City. Joseph J. Eller, 745 Fifth Avenue, New York 22, N. Y.

May 6-8: International Congress of Phlebology, Chambery, France. J. Marmasse, 3 Rue de la Republique, Orleans, Loiret, France.


May 23-28: Asian-Pacific Congress of Cardiology, Melbourne, Australia. A. E. Doyle, Alfred Hospital, Melbourne S. 1, Victoria, Australia.

August 14-20: Inter-American Congress of Cardiology, Rio de Janeiro. Secretariat, P. Box 1594, Rio de Janeiro, Brazil.

August 28-September 1: International Congress on Diseases of the Chest, Vienna. A. Sattler, American College of Chest Physicians, Frankgasse 8, Vienna, Austria.


September 18-25: European Congress of Cardiology, Rome. Secretariat, Clinica Medica, University of Rome, Italy.
SUNDAY MORNING

NOVEMBER 8, 1959

8:00 a.m. Registration

Aaron Kellner, Presiding

9:30

1. Influence of Dietary Carbohydrate and Protein on Serum and Liver Cholesterol in Germ-Free Chickens

David Kritchevsky, Ruth R. Kolman, R. M. Guttmacher and Martin Forbes. From the Wistar Institute of Anatomy and Biology, Philadelphia, Pa., and the Department of Microbiology, Temple University School of Medicine, Philadelphia, Pa.

It has been shown that specific dietary carbohydrates have a distinct effect on the serum cholesterol levels of rats, rabbits or chickens. Thus, animals on a sucrose-cholesterol diet exhibit higher serum cholesterol levels than do those ingesting glucose plus cholesterol. Addition of a sulfa drug or antibiotic to the diet does not affect the serum cholesterol level of the sucrose-fed animals, but causes a marked rise (30-40 per cent) in the glucose-fed groups. To further test this effect, conventionally reared and germ-free chickens were given diets containing 3 per cent cholesterol and differences in dietary carbohydrate (glucose and sucrose) and dietary protein (casein or soybean protein) were compared.

The germ-free state enhanced the growth of the glucose fed birds (215 vs. 195 Gm.) but not of the sucrose group (177 vs. 180 Gm.). The chickens fed casein grew better than did those fed the soybean, and the germ-free state resulted in better growth in both groups. Average weights in the casein group (348 vs. 305 Gm.) and in the soy protein group (233 vs. 218 Gm.). In general, liver lipid levels paralleled the serum cholesterol levels in all groups.

9:15

2. Effects of Carbohydrates on Fat Absorption

Alexander Michajlik and J. H. Bragdon. From the Section on Metabolism, National Heart Institute, Bethesda, Md.

It has been reported that the alimentary lipemia which follows a fat meal in man can be abolished by the concomitant feeding of glucose. It was suggested that the most likely explanation of this phenomenon was a more rapid removal of chylomicrons from the blood stream, but it had been previously reported from this laboratory that the rate of removal of injected chylomicrons was not significantly different in carbohydrate-fed rats compared with fasting controls. The effects of carbohydrate on fat absorption were therefore investigated in the rat.

Animals have been fed olive oil with and without starch, sucrose, and glucose. Five hours later the
AMERICAN SOCIETY FOR THE STUDY OF ARTERIOSCLEROSIS

9:30

3. Effects of MER-29 on Tissue and Plasma Cholesterol Concentrations and on Hepatic Cholesterol Synthesis from Mevalonic Acid

R. Gordon Gould, E. H. Lilly, and V. E. Mitchell. From the Los Alamos Scientific Laboratory, University of California, Los Alamos, N. M.

Blohm et al. have recently reported that MER-29 (1-[p-(β-diethylaminoethoxy)-phenyl]-1-(p-tolyl)-2-(p-chlorophenyl)ethanol) decreases cholesterol concentrations in rat plasma and tissue to remarkably low values and inhibits cholesterol biosynthesis as measured by incorporation of acetate-C¹⁴ in vivo. We have found the synthesis of cholesterol from mevalonic acid-2-C¹⁴ in rat liver homogenates to be inhibited to about 15 per cent of the control values after feeding 2.5 mg. of MER-29 per 100 Gm. rat per day for 2 days. Addition of 1 mg. of MER-29 to the homogenate from 5 Gm. of normal rat liver inhibited cholesterol biosynthesis from mevalonic acid by more than 90 per cent. These findings support the conclusion of MacKenzie and Blohm that the inhibitory effect occurs at a late stage, probably between some digitonin-precipitable sterol and cholesterol.

Male rats fed MER-29 at a level of 0.05 per cent of the diet for 42 days showed marked decreases (40-70 per cent), of cholesterol concentration in plasma, liver, adrenals, and intestine, and decreases of 20-30 per cent in the residual carcass values. Some slowing of weight gain was observed in younger rats but none in 300 Gm. rats. No other abnormalities or evidences of toxicity were observed.

Rats fed a diet containing 0.5 per cent cholesterol and 0.05 per cent MER-29 for 42 days gave cholesterol concentrations in plasma, liver, and other tissues almost as low as in rats fed MER-29 alone and far lower than would be expected in rats fed a 0.5 per cent cholesterol diet. These preliminary experiments suggest that MER-29 may be a powerful inhibitor of cholesterol absorption as well as an inhibitor of biosynthesis.

9:45

4. Observations Concerning the Site of Inhibition of Cholesterol Synthesis by MER-29

Mary L. Mobberley and Ivan D. Frenz, Jr. From the Cardiovascular Research Laboratory, Department of Medicine, Medical School, University of Minnesota, Minneapolis, Minn.

MacKenzie and Blohm have shown that when MER-29, 1-[(4-diethylaminoethoxy) phenyl]-1-(p-tolyl)-2-(p-chlorophenyl)ethanol, is given to rats, and the unsaponifiable lipids of the liver are labeled by administration of sodium acetate-1-C¹⁴, most of the radioactivity of the unsaponifiable lipids is eliminated by purification via the dibromide. Experiments were undertaken to localize more accurately the site of this block in cholesterol synthesis.

Rats were maintained for 10 days on daily doses of 5 mg. of MER-29 given subcutaneously as a suspension in olive oil. Slices of the livers were incubated for 1 hour in sodium acetate-1-C¹⁴, and the unsaponifiable lipids were chromatographed on silicic acid-Super-Cel. Radioactivity was associated with the various fractions from the column in the following percentages: squalene 12, lanosterol 15, 4-monomethylcholestenol 6, cholesterol 0, zymosterol-lathosterol 21, new higher counting companion, more polar than any of the above, 46. For an untreated control rat, the percentages were as follows: squalene 12, lanosterol 3, 4-monomethylcholestenol 2, cholesterol 58, zymosterol-lathosterol 25, new higher counting companion 0.

Liver homogenates from rats treated with MER-29 converted tritiated lathosterol to cholesterol in good yield, without the accumulation of tagged intermediates. The livers of treated rats accumulated large amounts of a sterol which behaved chromatographically and colorimetrically like zymosterol, as well as small but weighable amounts of the new companion. The block appears to involve the shift of the nuclear double bond from the 8 to the 7 position.

10:00

5. Comparison of a Direct Serum Procedure to Extraction Procedures for Measuring Serum Cholesterol

Robert V. Moore and Edwin Boyle, Jr. From the Lipid Metabolism Laboratory, Department of Medicine, Medical College of South Carolina, Charleston, S. C.
The many modifications of the classical Bloor method for determining cholesterol concentrations in serum with the consequential variance in "normal" results, plus the inherent errors of the many-step handling procedures for these extraction procedures led to a comparison of a direct procedure, with minimal handling procedures, to extraction procedures.

The direct serum cholesterol determination is a modification of the procedure of Zlatkis et al., with the color modification of Rosenthal et al., and will be presented briefly. The cholesterol values obtained will be compared to results obtained from extracted serum. The following facts have been established: 1. Cholesterol values obtained from extraction procedures tend to approach direct cholesterol values. 2. The difference in the values obtained by the various extraction procedures as done by the same personnel is small. 3. Multiple extraction procedures give higher values than single extraction procedures. 4. A high percentage of the cholesterol which is not extracted is usually found in the preparative ultracentrifuge fraction with a density > 1.063 Gm. per ml., but proteins in other lipoprotein components may, and do at times, hold their lipid moieties so firmly as to prevent their complete extraction. 5. The unique character of the sera of individuals, as shown by a variability of extractability from 50-100 per cent, each individually reproducible, is clearly set forth. 6. The errors inherent to hemolysis and to physiologic concentrations of bilirubin, niacin acid, and the fat soluble vitamins A, D, E, and K have been studied and have been found negligible under normal conditions. These facts show that values obtained from direct serum determinations are more likely to reflect the cholesterol moiety of serum. The direct serum determination method gives more consistent, reproducible, and reliable results. The direct test described is also much simpler, much more rapid, and less expensive to perform.

10:15
6. Comparative Quantitation of the Sources of Plasma Cholesterol in Dog and Man

Richard Abbuhl, C. Bruce Taylor, Dorothy Patton and George E. Coz. From the Department of Pathology, Presbyterian- St. Luke's Hospital, Chicago, Ill.

Suppression of hepatic, but not extrahepatic, cholesterol synthesis by dietary cholesterol, justifies considering these as separate sources of plasma cholesterol. Quantitation of their relative contributions is being attempted as follows: By continued administration of a diet containing large, known amounts of cholesterol, hepatic cholesterol synthesis is virtually eliminated in all species that have been adequately studied. Using labeled dietary cholesterol with known specific activity, the ratio of plasma cholesterol specific activity to dietary cholesterol specific activity will rise to a maximum which represents the fraction of plasma cholesterol derived from diet. This should equal the maximum hepatic contribution when no cholesterol is ingested. The remaining fraction of plasma cholesterol must derive from extrahepatic cholesterol synthesis. Morris et al. applied this principle to rats. We are applying it to man and other species. In dogs, the greatest contribution of dietary cholesterol to plasma cholesterol is obtained when egg yolk is used as a medium for administration; upon the continued administration of 3 Gm. cholesterol per day, about 90 per cent of plasma cholesterol comes from the diet. With human subjects, 5 Gm. cholesterol per day dissolved in margarine supplied 10 to 15 per cent of plasma cholesterol; higher percentages are expected from the study now in progress, where egg yolk is used instead of margarine.

10:30
7. On the Interpretation of Disappearance Curves of Radioactive Serum Cholesterol

Joel Avigan and Daniel Steinberg. From the Section on Metabolism, National Heart Institute, Institutes of Health, U.S. Public Health Service, Bethesda, Md.

A number of authors have measured the disappearance of fed or injected radioactive cholesterol from the serum compartment and have assigned a rate constant to this disappearance. More recently it has become clear that this disappearance is not a true first order process and therefore the "half life" of serum cholesterol is not a meaningful term. These studies in rats were undertaken to evaluate exchange processes between serum cholesterol and tissue cholesterol in various organs over extended time periods. Tracer doses of 4-C\textsuperscript{14} cholesterol were fed to rats which were then sacrificed at intervals from 6 hours to 7 weeks. The specific radioactivity of cholesterol in the liver and small intestine paralleled that of the serum quite closely throughout the study. On the other hand, the specific radioactivity of cholesterol in the skeletal muscle, kidney and brain, while initially very low relative to that of the serum, was considerably higher at 7 weeks. By that time the ratios of specific activities of cholesterol in these tissues relative to that in serum were, respectively, 3.3, 5.9, and 1.8. The ratios
8. Lability of Cholesterol in Human Atherosclerotic Plaques

R. Gordon Gould, Richard J. Jones, and Robert W. Wissler. From the Los Alamos Scientific Laboratory, University of California, Los Alamos, N. M., and the Departments of Medicine and Pathology, University of Chicago, Chicago, Ill.

A previous report demonstrated that dietary cholesterol mixes with and becomes indistinguishable from the cholesterol of endogenous origin in the liver-blood pool. The cholesterol in this pool equilibrates with that in all tissues studied in experimental animals, except brain, but at widely varying rates. The question arises to what extent the cholesterol present in human atherosclerotic plaques is still capable of equilibration with blood cholesterol, since this will be an indication of the reversibility of the atherogenic process.

Tritium-labeled cholesterol was fed to a group of patients with poor prognoses (including some with myocardial infarction) at a constant daily dosage after a priming dose 10 times as large. This regimen was found to give almost constant plasma cholesterol specific activity values. The cholesterol concentration and specific activity were determined in samples of plasma, liver, the coronary, iliac and pulmonary arteries, samples of several parts of the aorta, and of various other tissues from all patients coming to autopsy. Results were generally in agreement with previous animal studies in showing most rapid equilibration of liver-blood cholesterol with spleen, intestine, lung, heart, adrenal, and kidney; less rapid with skeletal muscle and fat; and slowest with arteries. The more severely atherosclerotic samples of arterial tissue showed, in general, slower rates of equilibration but even those with cholesterol concentrations over ten times the normal values gave, in 1 case, 8 per cent equilibration in 4 days (mean of 3 samples) and, in a second case, 11 per cent in 14 days (mean of 3 samples).

One patient who took cholesterol-H³ daily for 2 months and who died 5 months after the last dose showed essentially equal specific activity values in serum, liver, spleen, and thoracic aorta and 75 per cent of this value in abdominal aorta. Atherosclerosis score was 2+ in thoracic and 3+ in abdominal aorta. These results suggest that the cholesterol in human atherosclerotic lesions is interchangeable with blood cholesterol but at a very slow rate.

11:00

9. Origin of Various Lipids in Atheromatous Lesions of Rabbits

H. A. Newman and D. B. Zilversmit. From the Department of Physiology, University of Tennessee, Memphis, Tenn.

The finding that plaque phospholipid was synthesized by the arterial wall is based on studies with P³². Since incorporation of P³² does not necessarily provide a valid index of synthesis of the fatty acid portion of the phospholipid molecule nor give any information on the synthesis of other lipids, C¹⁴-acetate was employed in the present study. Albino rabbits on 1 per cent cholesterol intake for 3-4 months were either eviscerated (to suppress plasma-lipid synthesis) or sham operated. Thirty minutes to 5 hours after injection of C¹⁴-acetate tissues were sampled for lipid analysis. All animals exhibited gross atheromatous lesions. The specific activity of atheromatous phospholipids in the eviscerates exceeded that in plasma in all animals by factors ranging from 10 to 74. Thus the C¹⁴ studies confirm earlier findings with P³² that a major portion of the labeled arterial phospholipid derives from synthesis in situ. Triglyceride specific activities in aorta also exceeded those in plasma, but cholesterol specific activities were too low to give reliable information. To trace the origin of aortic cholesterol several normal rabbits were placed on a constant intake of 1 per cent C¹⁴-cholesterol in their diet until different degrees of atheromatosis were established. Multiple determinations of the specific activity of serum cholesterol and a final determination of aortic cholesterol specific activity were performed. Preliminary results indicate considerable deposition of plasma cholesterol or exchange between plasma and aortic cholesterol takes place.
10. Influence of Cholesterol Concentrations on Biosynthesis of Proteins in the Isolated Perfused Rat Liver

Paul S. Roheim, David E. Haft, Abraham White, and Howard A. Eder, From the Departments of Medicine, Biochemistry, and Radiology, the Albert Einstein College of Medicine, New York, N. Y.

It has been shown that the plasma lipoproteins can be synthesized both by rat liver slices and by the isolated perfused rat liver. Previous studies using the perfused rat liver have shown that prefeeding the liver donor rat with cholesterol results in marked inhibition of cholesterol biosynthesis but no inhibition of protein synthesis as measured by incorporation of uniformly labeled lysine C\(^{14}\). X-radiation, 2400 r., to the liver donor rat resulted in an appreciable increase in cholesterol synthesis, but its effects on the synthesis of proteins in the lipoprotein fractions were less marked.

Measurements of lysine incorporation into the proteins of the high (D > 1.063) and low density (D < 1.063) lipoprotein fractions by rat livers perfused with plasma obtained from rats fed a high cholesterol diet for 13 days have been made. The cholesterol content of the perfusate was twice that in the control perfusates, and this was associated with an increase in the concentration of low density lipoproteins in the perfusate. In the three experiments completed, the specific activities of the high and low density lipoproteins were within the normal range, but the total incorporation of C\(^{14}\) was increased in the low density fraction but not in the high density fraction. Further investigation of the effects of altering the lipid content of the perfusion fluid are in progress.

11:30

11. Identification of Substances Responsible for Lipemia-Producing Activity of Pituitary Gland

Daniel Rudman, Floyd Seidman, and Maria B. Reid. From the Columbia Division, Goldwater Memorial Hospital, and Department of Medicine, Columbia University, New York, N. Y.

Previous reports from this laboratory have shown that a single injection into rabbits of unfraccionated alkaline extract of 30-90 mg. of desiccated hog anterior pituitary lobes consistently produces a two to fivefold increase in serum lipids. No effect upon serum lipids is produced by the 6 recognized anterior lobe hormones when tested separately at doses equivalent to 500 mg. of the gland. However, a combination of ACTH with any of the other 5 hormones causes lipemia in about 50 per cent of rabbits when tested at doses equivalent to 200 mg. of gland. Combinations of hormones not including ACTH have no effect upon the rabbit's serum lipids.

Fractionation of crude pituitary extract by the method of Bonsnes shows that the major portion of the lipemia-producing activity can be separated from ACTH, growth hormone, and lactogenic hormone. Hog pituitary glands are extracted with 2 per cent NaCl solution at pH 7.6. The extract is adjusted to pH 4.5 and the precipitate removed. Fractional precipitation with acetone is then carried out. The major portion of the lipemia-producing activity is concentrated in the fraction precipitated between 75 per cent and 90 per cent acetone concentration. This material is further purified on an ion-exchange column. The final fraction has a lipemia-producing potency about 10 times greater than that of desiccated hog anterior pituitary lobes, and 7 times greater than that of the unfraccionated anterior lobe extract.

ACTH has been shown to have a potentiating effect upon the production of lipemia. In addition, these observations suggest the existence of a separate lipemia-producing pituitary hormone.

11:45

12. Fecal Steroid Analyses in Man

R. S. Rosenfeld and Leon Hellman. From the Sloan-Kettering Institute for Cancer Research, New York, N. Y.

The major route for the elimination of cholesterol from the body is fecal excretion in the form of C-27 steroids and bile acids. The relative quantities of neutral steroids as compared to bile acids and the composition of the neutral steroids are of importance in investigation of mechanisms by which hypocholesteremic agents achieve their effects. Coprostanol, the principle fecal sterol, was presumed to be formed by the reaction pathway (1) cholesterol → cholestenone → coprostanone → coprostanol, but more recently we have shown by double-labeling techines that the direct reaction (2) cholesterol → coprostanol is more likely. The analytic data to be presented, representing 55 determinations in 14 subjects on modern U.S. diets, provide further information concerning these two pathways.

The dry weight of feces was 9-56 Gm. (average 25) per day. The steroids were in the unconjugated form and constituted 4-10 per cent of the dry weight. The steroids were composed of 43-85 (average 66) per cent neutral steroids and 15-57 (average 34) per cent of bile acids. Chromato-
graphic separation of the neutral steroid fraction gave the following results: coprostanone, 0.4-11 per cent (average 6 per cent); coprostanol, 45-82 per cent (average 64 per cent); and cholesterol, 5-25 per cent (average 11 per cent). The cholesterol fraction contained 5-30 per cent (average 20 per cent) cholesterol. Separation of both the coprostanol and the cholesterol fractions into α and β sterols showed that over 90 per cent was contained in the β fraction. Cholestenone could not be detected. During the hypocholesteremic effect of corn oil, there was no increase in the excretion of either α sterols or bile acids, but a significant increase in the β sterol fraction. The absence of cholestenone may be taken as further evidence for pathway (2) or suggests that it may be in a very rapidly turning-over pool of minute size in pathway (1).

SUNDAY AFTERNOON

12:00 Luncheon
G. Lyman Duff Memorial Lecture:
Dr. Paul Dudley White, "Atheroma and Thrombosis—Major Threats to Our Health Today."

2:00 Business Session
3:00 Presidential Address:
James C. Patterson

Forrest E. Kendall, Presiding
3:30
14. Effect of Rate of Ingestion of the Diet ("Meal Eating" vs. "Nibbling") on Atherogenesis in Chickens
Clarence Cohn, Ruth Pick, and Louis N. Katz, with the assistance of Louisea Bell, Phillip Johnson and Dolores Century. From the Departments of Biochemistry and Cardiovascular Research, Michael Reese Hospital, Chicago, Ill.

Evidence suggesting that the rate of ingestion of the diet plays a significant role in the regulation of intermediary metabolism, and as a result, in over all body metabolism, has been summarized recently. As part of a long-term study of the metabolic alterations produced by changing nibbling animals to meal eating ones, the effect of these feeding habits on atherogenesis was evaluated in chickens. One group of birds was allowed to eat ad libitum ("nibbling") a diet containing 0.5 per cent cholesterol, 5 per cent cottonseed oil and 20 per cent protein and another group was presented with this diet for an hour in the morning and an hour in the afternoon ("meal eating"). After 5 experimental weeks, the animals were bled for serum lipid analyses and sacrificed for evaluation of gross aortic and microscopic coronary atherosclerosis. The "meal eating" chickens ate about one third less diet and gained about one third less body weight than the "nibblers." Mean serum cholesterol levels of the nibbling birds, which had eaten all night prior to sacrifice, was 294 mg. per cent; by contrast, the serum cholesterol of the "meal eaters," bled 18 hours after their last meal, was 584 mg. per cent. Grossly, the thoracic aortas of the "meal eaters" exhibited both an increased incidence (100 vs. 44 per cent) and severity (1.80 vs. 0.42) of atherosclerotic lesions. Microscopically, coronary atherosclerosis was present in 2.2 per cent of the vessels of the "nibblers" and in 14.6 per cent of the vessels of the "meal eaters." The results are interpreted to indicate that the rate of ingestion of the diet plays a role in atherogenesis.

3:45
14. Reduction of Plasma Cholesterol in Animals with Bile Acid Sequestrants

David M. Tennent, Henry Siegel, Mary E. Zanetti, Gunther W. Kuron, Walther H. Ott, and Frank J. Wolf. From the Merck Institute for Therapeutic Research, Rahway, N. J.

Present evidence indicates that the rate of oxidation of cholesterol is regulated by the need for bile acid in the enterohepatic cycle. Removal of bile acid from the enterohepatic cycle by introduction of a bile acid sequestrant into the intestine should increase the oxidative destruction of cholesterol.

We have studied the effect of feeding 2 high molecular weight polymeric quaternary ammonium salts on blood cholesterol in experimental animals. These compounds form insoluble or undissociated complexes with bile acids, but are not themselves digested or absorbed from the gut.

In short-term experiments plasma cholesterol rise in cholesterol-fed cockerels was inhibited 50 per cent by feeding these compounds as 1 per cent of the diet. In longer experiments cholesterol levels and plaque formation also were similarly reduced. In normocholesterolemic cockerels, fed basal diet, cholesterol concentrations were reduced from an average of 73 mg. per cent to 51 mg. per cent in 4 days.

In normal dogs cholesterol concentrations were reduced 25 per cent by sequestrant feeding from control levels near 100 mg. per cent. In experiments lasting as long as 1 year dogs fed sequestrants daily appeared to be alert and free from toxic effects. Hematological and biochemical tests made at intervals yielded normal results, except
for lowered plasma cholesterol levels. There were no signs during life or at autopsy of impaired fat absorption. At autopsy no pathologic changes attributable to sequence feeding were observed either grossly or on microscopic examination of the tissues.

4:00
15. Response of Man to Dietary Cholesterol

J. M. R. Beveridge, W. F. Connell, G. A. Mayer and H. L. Haust. From the Departments of Biochemistry and Medicine, Queen’s University, Kingston, Ontario, Canada.

In 1958 we reported at the IVth International Congress of Biochemistry that one of the factors responsible for the hypercholesterolemic activity of butter fat was its cholesterol content. Further work utilizing homogeneous formula diets has demonstrated that the serum cholesterol level in man is in fact highly responsive to the concentration of dietary cholesterol. Ninety-three subjects (undergraduate students) were placed upon a homogenized fat-free diet for a period of 8 days at which time they were divided into 8 groups and given a diet modified by the substitution of a butter oil fraction for 30 per cent of calories at the expense of carbohydrate. The butter oil fraction represented approximately 60 per cent of the original butter, the more volatile triglycerides and essentially all of the cholesterol having been removed by distillation in high vacuum, a process very kindly carried out for us by the Distillation Products Industries. The amounts of added purified cholesterol in milligrams per 950 calories were as follows: Group 1, nil; 2, 25 mg.; 3, 50 mg.; 4, 100 mg.; 5, 200 mg.; 6, 400 mg.; 7, 800 mg.; and 8, 1,600 mg. Sixty-seven subjects successfully completed the experiment. The serum cholesterol values were found to increase with increasing dietary cholesterol up to between 200 and 400 mg. per 950 calories which corresponded to about 600-1,200 mg. per day. At dietary cholesterol levels of above 400 mg. per 950 calories the serum concentrations of the sterol plateaued. On the basis of these experiments there can no longer be any doubt of the responsiveness of man to dietary cholesterol although the nature of the accompanying fat or oil affects to a large extent the response obtained.

4:15
16. Cholesterol Metabolism in Methyl Testosterone-Treated Dogs

Liese L. Abell, E. H. Mosbach, and F. E. Kendall. From the Columbia University Research Service, Goldwater Memorial Hospital and the Departments of Biochemistry and Medicine, College of Physicians and Surgeons, Columbia University, New York, N. Y.

In 1957 Furman reported that oral administration of methyl testosterone (50 to 600 mg. per day) to dogs consistently lowered their serum cholesterol levels. We have studied the effect of methyl testosterone in dogs maintained on diets containing added fat and/or added cholesterol. When 200 mg. per day of methyl testosterone was given to dogs on low cholesterol diets providing 40 per cent of the calories as fat, the serum cholesterol levels dropped 51 per cent. In contrast, on high fat diets containing 2.5 per cent cholesterol, this dose of methyl testosterone increased serum cholesterol levels 39 per cent. Balance experiments were carried out in 2 dogs maintained on a stock diet. After a suitable control period the animals received 50 mg. per Kg. per day of methyl testosterone orally for 3 weeks and then were taken off the drug for another control period. Feces were collected daily throughout the experimental periods and analyzed for steroids and bile acids.

It was found that on the low fat, low cholesterol diet, the cholesterol excretion did not change in response to methyl testosterone administration. The bile acid excretion tended to rise during the period of drug administration, but did not return to control levels after the drug was withdrawn. Additional studies with dogs receiving diets high in fat and/or cholesterol are in progress and will be discussed.

4:30
17. Comparison of Effects of Butter and Egg Yolk on Development of Atherosclerosis in Swine

H. C. Rossell, H. G. Downie, and J. F. Mustard. From the Ontario Veterinary College, Guelph, and Department of Medicine, Sunnybrook Hospital, University of Toronto.

An earlier study showed that swine develop some atherosclerosis on low fat diets. A diet containing 40 per cent of the calories as margarine does not accelerate the development of atherosclerosis while a diet containing 40 per cent of the calories as butter does. A further study has been done comparing the effects of egg yolk and butter on the development of atherosclerosis.

Thirty-three swine were divided into 3 matched groups of eleven. One group was maintained on the basic diet, a second group on the basic diet plus 33 per cent of calories as butterfat, a third group on the basic diet plus 33 per cent of calories as egg yolk. All diets were isocaloric. The animals were studied at intervals during the experiment. The indicies followed were: Clearing factor activ-
18. Effects of Material Rich in Phosphatidyl Ethanolamine or Phosphatidyl Serine on Clotting and Cholesterol Levels

J. F. Mustard, H. G. Downie, H. C. Rowsell, and A. Bier. From the D.V.A. Sunnybrook Hospital, Toronto, Department of Medicine, University of Toronto, and Ontario Veterinary College, Guelph.

Fractions rich in phosphatidyl ethanolamine (PE) and rich in phosphatidyl serine (PS) were prepared from beef, swine and human brain and liver by modifications of Folch's techniques. Using the thromboplastin generation test, PE was active in thromboplastin formation, while PS over the same range of dilution was inactive. PS in concentrations 0.1 that of PE inhibits the activity of the latter phospholipid in this test. Addition of PE to blood as it clots accelerates the rate of loss of AHG activity, and thrombin formation while addition of PS slows the rate of fall in AHG activity, and thrombin formation. These in vitro studies indicate that PE rich material accelerates clotting and PS rich material inhibits clotting.

The in vivo clotting effects were studied using swine. Intravenous PE increased Christmas factor (PTC) activity, decreased AHG activity and the circulating platelet count, accelerated the Russell viper venom time (RVVT) and clotting time. Intravenous PS decreased Christmas factor activity, prolonged the clotting time and prothrombin time, but accelerated the RVVT. There was little change in the platelet count and AHG activity. Therefore, intravenous PE accelerates the thromboplastin mechanism, whereas PS inhibits this mechanism.

PS appears to inhibit Christina's factor and factor VII activity. Since the RVVT is accelerated in vivo under the conditions where there is an anticoagulant effect, some doubt is cast on the validity of this test as an index of accelerated clotting.

Administration of 5 to 15 Gm. per day of PS rich material to 5 subjects receiving 35 per cent of their calories as animal and vegetable fat lowered the cholesterol level by 10 to 20 per cent. PE had no effect. Therefore, PS rich material can have as well as an anticoagulant effect a hypocholesterolic effect.

Robert H. Furman, Presiding

9:00

19. Histochemical Studies of Cerebral Arteries

Frederick T. Zugibe and Kenneth D. Brown. From the Geriatrics Research Project, Veterans Administration Hospital, Downey, Ill.

Histochemical studies of the cerebral arteries have been grossly neglected. The following is a preliminary report of our observations on grossly normal and early lesions of cerebral arteries of individuals ranging from fetuses to 65 years of age. Newer embedding techniques utilizing carbowax which have been recently developed in our laboratory produce sections with minimal lipid loss, allow routine stains to be applied to adjacent sections, and further permit the observations of both lipid and acid polysaccharide on the same tissue section.

There is no relationship between acid mucopolysaccharides (AMP) and lipids in respect to staining intensity and/or distribution in any of the age groups studied.

Lipid was essentially absent in the fetuses. Occasionally slight lipid tinting was observed in the internal elastic membrane of juveniles. The most striking observation in the adults was the presence of lipid in the internal elastic membrane and reduplication of elastic elements in the intima.

Data from adjacent sections cut at 1 µ and stained for lipid supported our previously reported observation on aortas that the lipid was within the fiber and not physiologically oriented on the outside surface. In fetuses and infants there was an increase in testicular hyaluronidase hydrolyzable AMP principally in the proximal medial surface. In the adults there was an increase in testicular
hyaluronidase resistant AMP in the proximal media and in the intima. This increase in AMP corresponded to the areas of collagen increase. Fragmentation, fraying, and reduplication of the internal elastic membrane was rarely present in the fetuses and infants. This would suggest that there is no relationship between the presence of AMP and these elastic changes.

In adults, reduplication of the internal elastic membrane was frequently observed principally in the region where the intima was thickest. The significance of these observations will be discussed.

9:15

20. Vascular Lesions Induced by Serotonin and Adrenalin

Takio Shimamoto. From the Department of Clinical Physiology, Tokyo Medical and Dental University, Yushima, Bunkyo-ku, Tokyo, Japan.

Recently, we succeeded in producing a fibrinous type of atheroma with regular complications of myocardial infarct-like lesions, and encephalomalacia in rabbits. Cerebral hemorrhage occurred from venules located specifically in the thalamo-striatal region. These hemorrhages closely resemble cerebral hemorrhage seen in hypertensive patients. These lesions are being produced by combinations and interaction of serotonin and adrenaline. These lesions are produced with great regularity.

9:30

21. Nature of Lipid Material in Involved Areas of Human Aortas

M. Sugai, T. Nishida and F. A. Kummerow. From the Department of Food Technology, University of Illinois, Urbana, Ill.

Lipid material which could not be extracted with fat solvents was found in the involved, but not in the uninvolved portions of the intima stripped from human aortas. This lipid material could only be freed by mild saponification with alkali.

The lipid material in symplexes, which are formed in vitro by the interaction of various proteins such as blood serum or a solution of egg albumin and oxidized oil, could also not be removed by solvent extraction but could be freed by saponification with alkali, thus showing a similarity in properties between the lipids extracted from involved areas of the intima and the lipids in symplexes.

We, therefore, studied the interaction between fatty peroxides and the β-lipoproteins in human blood. The results indicated that the analytical centrifugal pattern of the β-lipoproteins in human blood became more heterogeneous after contact with fatty peroxides. A gradual increase in the S₇ value was noted and the area under the lipoprotein peak gradually decreased. The chemical nature of this interaction and symplex formation will be discussed.

9:45

22. Composition of Fatty Acids in Cholesterol Esters Derived from Normal and Abnormal Intima


Areas of normal and abnormal tissue were first delineated then cut from human aortas. The intima was stripped from the media. The pools of medial and intimal tissue were extracted separately. The cholesterol esters were isolated, purified, and then hydrolysed. The fatty acids were analyzed by gas liquid chromatography.

Material from 1 young woman and an older man has been analyzed at the time of submission of this abstract. There is no significant difference in the fatty acids of the cholesterol esters of the media; whereas significant differences are seen in the intima.

These can be grossly described as: 1. A higher proportion of the longer chained fatty acids are found in esters from abnormal tissue than in those from normal tissue. 2. Although they are minor components; an absence of acids shorter than C14 in cholesterol esters from abnormal tissue is seen as compared with the presence of such acids in normal intima. 3. A marked drop (from 18 per cent in normal tissue, to 3 per cent in abnormal) in the linoleic acid percentage composition was seen in the older patient whereas there was no difference seen in the younger patient between abnormal and normal intima. 4. The presence in 1 patient of a significant percentage (17 per cent of a presently unidentified long-chained (C20 or more) multisaturated fatty acid in the esters derived from the abnormal tissue. This acid if present in normal tissue could only have been present in very minute quantities.

These data are sufficient to show that the cholesterol esters derived from abnormal intima are chemically different from those derived from the normal intima of the same individual. It is expected that further analyses, perhaps sufficient to show an age trend will be available for presentation at the meeting.
10:00

23. Further Studies on Spontaneous Atherosclerosis in Pigeons

H. B. Lofland, T. B. Clarkson, R. W. Prichard and M. G. Netsky. Winston-Salem, N. C.

The White Carneau, Silver King, and Autosexing King pigeons develop spontaneous atherosclerosis on grain diets, unsupplemented with fat or cholesterol. Grossly and microscopically, the lesions resemble those of human beings. Show Racer pigeons, maintained under identical conditions, were practically free of atherosclerosis.

Six to 9-months-old White Carneaux were free of atherosclerosis, whereas 66 per cent of 12-month-old birds had lesions. Aortic plaques were observed in 59 per cent of the 18-24-month-old birds. All birds over 36 months of age were atherosclerotic. No differences in levels of serum cholesterol, phospholipid or total lipid were observed in the various age groups. The levels of aorta cholesterol rose strikingly at 24 months.

When young pigeons of resistant and susceptible breeds were fed fat and cholesterol, onset of the disease was accelerated in susceptible breeds; non-susceptible breeds appeared more resistant to cholesterol feeding.

The extent of coronary atherosclerosis was determined by examining sections at intervals of 200 µ from the apex to the base of the heart of 20 7-year-old White Carneaux; 70 per cent had coronary artery lesions, with the same morphologic components as aortic lesions.

When isolated aortas from resistant and susceptible breeds were incubated in vitro with acetate 1-C14, radioactivity was recovered in the total lipid extract, and in a digitonin-precipitable material. The two breeds were similar in amounts of acetate incorporated. In most cases the distal end of the aorta showed a higher rate of acetate incorporation than the proximal end.

10:15

24. Breed Susceptibility in Rabbits to Hypercholesterolemia and Atherosclerosis

DeWitt Hendee Smith and Elizabeth Gaman. From the New Jersey Neuro-Psychiatric Institute, Princeton, N. J.

Investigators using rabbits in atherosclerosis research often are annoyed at the variation of response encountered. Reports seldom state the breed of rabbit used. To assess this factor we fed 1 per cent cholesterol for 4 weeks to litters of different rabbit breeds and hybrids. Results have been so unexpected and striking that we are making this preliminary report.

Within litters cholesterol variation was itself variable, so that in Flemish Giant and New Zealands cholesterols were highly scattered. In the other litters cholesterols were more closely grouped. The mean cholesterols, distributed more normally, ranged from 749 ± 293 for the Flemish to 1636 ± 182 for the New Zealand-Californian cross.

The kind and extent of atherosclerosis gave some surprises. All but 2 of the litters showed typical atheromatous plaque deposits in the aorta varying somewhat within and between litters. Among these the New Zealands showed more and the Californian and New Zealand-Californian hybrids and intermediate atheroma, the chinchillas somewhat less. On the basis of amount or uniformity, there was no great preference. However the Flemish with low and highly variable cholesterol levels, showed a uniform wrinkling of the aortic intima, seemingly unique to this breed, which was confirmed microscopically as thin lipid deposit, but no plaques were present. The Dutch, with the least variation in cholesterol level, showed no aortic change whatever. The New Zealand-Californian hybrid had the highest cholesterol, low variability, good plaque formation and many other laboratory advantages.

Hybridization with selected parents may prove a useful way in which to cut down rabbit variability.

10:30

25. Cholesterolemia, Occupation, Physical Activity and Diet in the Middle-Aged Employees of a Chicago Utility Company

Jeremiah Stamler, Howard A. Lindberg, David M. Berkson, Wilda A. Miller and Marilyn Pagannin. From the Heart Disease Control Program, Chicago Board of Health and the Department of Medicine, Northwestern University Medical School, Chicago, Ill.

During 1958, analyses of serum cholesterol were accomplished on over 90 per cent of the labor force aged 40-59 of a Chicago utility company. The mean values for 1,859 men and 170 women were 237 and 241 mg. per cent respectively. A minority of both sexes had values below 225 mg. per cent. Twenty and 25 per cent of the men and women respectively had values of 275 mg. per cent or greater; 9 per cent and 14 per cent, of 300 mg. per cent or greater. Little or no correlation was observed between cholesterol and weight-blood pressure levels. When the male population was stratified based on occupational and other sociological criteria—e.g., professional-executive-managerial-supervisory-technical personnel; clerical-sales; foremen; skilled-semiskilled and unskilled laborers; sedentary and nonsedentary employees;
salary and wage; indoor and outdoor; white- and blue collar; more and less educated; veteran and nonveteran—differences in cholesterol levels for the subgroups were generally insignificant. Prevalence rates for obesity and hypertension were also similar in the subgroups, as were patterns of diet and physical activity off the job. These data are consistent with the findings on occurrence rates of coronary heart disease in these subgroups of middle-aged working men.

10:45
26. Epidemiology of Atherosclerotic Lesions

Henry C. McGill, Jr., Jack P. Strong, Russell L. Holman, and C. A. McMahon, Louisiana State University, New Orleans; Carlos Tejada, Instituto de Nutricion de Centro America y Panama, Guatemala; Carlos Restrepo, Universidad del Valle, Cali, Colombia; Egon Lichtenberger, Hospital San Juan de Dios, Bogota, Colombia; and Lorenzo Galindo, University of Puerto Rico, San Juan, Puerto Rico.

Quantitative evaluations of coronary and aortic atherosclerotic lesions were compared in males 20-59 years necropsied in 4 geographic areas and representing 5 different population groups. Differences in fatty streaks from 1 group to another (maximum variation, twofold) were much less than variations in fibrous plaques (variation up to fivefold). For example, in coronary arteries 40-59 years of age, the average percentages of intimal surface covered by fibrous plaques were as follows: New Orleans white, 16; New Orleans Negro, 13; Puerto Rican, 10; Colombian, 4; and Guatemalan, 4. These values for coronary fibrous plaques are strongly correlated with percentages of natural deaths due to ischemic heart disease in the various necropsy groups, while values for fatty streaks are not. Thus the earliest stage of atherosclerotic lesions that can be statistically associated with ischemic heart disease on a group basis is the fibrous plaque. These and previous studies lend support to the idea that fatty streaks (as measured in this manner) are only slightly influenced by environment, while fibrous plaques may be predominantly determined by environment. The possibility remains that the qualitative lipid composition of fatty streaks, which may be environmentally determined, could account for the differences in fibrous plaques.

11:00
27. Vascular Lumen and Blood Flow

Simon Rodbard, From the University of Buffalo Chronic Disease Research Institute, Buffalo, N. Y.

These studies were undertaken to examine mechanisms involved in the regulation of the cross-section area of vessels. A single carotid artery was completely ligated in dogs and rats. Findings in the untouched contralateral artery were of special interest: Within 3 days an area of the internal elastic membrane became fragmented, and macrophages containing elastica-staining material became evident at this site; collagen fibrils filled the are, and an endothelial "plaque" appeared at the site. The ligated arteries showed progressive reduction of the lumen as a result of subendothelial proliferation and endothelial infolding. These findings may be interpreted as follows: Blood flowing through a vessel produces a mechanical drag on the endothelium, in accord with the velocity of the stream. Ligation of one carotid artery tends to increase the volume and velocity of blood flow in the contralateral carotid. The increased drag on the endothelium leads to lysis of an arc of the internal elastic membrane, followed by reconstruction of the vessel around a larger lumen in which the flow velocity and drag are returned to normal levels. Diminution in flow reduces velocity and drag; this is followed by subendothelial proliferation, infolding of the lining, and ultimate reorganization of the vessel around a reduced lumen. These findings suggest that the velocity of blood flow and the consequent drag on the boundary layer between blood and lining may play a role in the regulation of the cross-section area of blood vessels.

11:15
28. Thrombotic and Inflammatory Origin of Arteriosclerosis

Robert H. More and M. Daria Haust, From The Department of Pathology, Queen's University, Kingston, Ontario, Canada.

There is evidence in man and animals of a relation between serum lipids and the accumulation of lipid in the intima of the aorta and its immediate branches. It is however, not so apparent that there is such a relation between serum lipids and the pleomorphic intimal disease traditionally designated by the term arteriosclerosis. Some 1,300 sections of lesions from 150 aortas and the coronary arteries of 100 hearts, were examined in this study. A variety of stains and histochemical procedures were carried out on additional slides cut from these sections. In some instances it was apparent that the lesions did not start from a deposit of grossly or microscopically visible lipid. Many lesions were initiated and many progressed by means of inflammation of the intima and thrombosis. Progression was characterized by fibrosis of these lesions and repeated episodes of inflammation and thrombosis. These studies were not designed to evaluate the role of serum lipids in the
initiation and progression of these lesions. However if serum lipids are important in the etiology and pathogenesis of any or all stages of the pleomorphic disease arteriosclerosis, then it would appear that one of the ways they act is by producing either inflammation, or thrombosis or both.

11:30

29. Papain Atherosclerosis

Fiorenzo Paronetto and David Adlersberg. From The Mount Sinai Hospital, New York, N. Y.

The well-established effects of papain on the ground substance suggested a study of prolonged papain administration upon the arterial tree in old and young rabbits. The old group consisted of 20 “old breeders,” approximately 3 years old with a body weight of 4-5 Kg. The young group included 7 animals approximately 1-month-old with body weights ranging from 1.2 to 1.7 Kg. Each animal received 3 times weekly an intraperitoneal injection of papain in physiologic saline solution. The duration of the experiments varied from 15-60 days. All animals received Purina chow and water ad lib.

Fifty per cent of the “old breeders” presented unusual changes in the aorta consisting of slightly elevated white plaques varying from 0.2 to 0.3 cm. in diameter. Some of these plaques were isolated and discrete while others showed confluence which resulted in longitudinal streaks and larger areas of atheroma-like appearance. The plaques were seen in the ascending aorta, in the arch and, in the severe cases, in the distal aorta to the bifurcation of the iliae arteries. Although considerable variations were seen among the animals, the severity of the lesions seems to parallel the duration of papain administration. Two animals showed dissecting aneurysms of the aorta with hemorrhage into the wall. In contrast to the “old breeders,” the “young” group showed no gross aortic lesions.

Histologically, the lesions presented severe changes in the media and intima with accumulation of metachromatic material and calcium but without any accumulation of lipid. Extensive fragmentation and coarsening of the elastic fibers were seen.

Thus, prolonged parenteral administration of papain produced striking gross and histological changes in the aorta in old rabbits.

11:45

30. Fatal Myocardial Infarction in Rhesus Monkeys with Diet-Induced Hypercholesterolemia

C. Bruce Taylor, George E. Cox, Marjorie Counts and Nelson Yogi. From the Department of Pathology, Presbyterian-St. Luke’s Hospital, Chicago, Ill.

In earlier studies we reported the development of atheromas in the coronary arteries and the aorta and its major branches in Rhesus monkeys when mean cholesterol levels were maintained between 256 and 393 mg. per cent for 6 months or more. Hypercholesterolemia was induced by feeding an adequate diet containing 22 per cent butter fat and supplying 1.5 Gm. of cholesterol per day. Two female animals on this diet showed a marked propensity for hypercholesterolemia. They were maintained on this diet for 3 and 4 years and had mean serum cholesterol levels of 659 and 554 mg. per cent. Both animals developed cutaneous xanthomas on the hands and feet. One animal died from massive myocardial infarction. Five thrombotic occlusions showing various stages of organization were found in the coronary arteries. The coronary arteries also showed marked athero-arteriosclerotic narrowing, medial degeneration with fibrosis, calcification or foam cell infiltration, markedly increased medial vascularization and focal areas of secondary arteritis. The similarity to human coronary arteriosclerosis was striking. The other animal developed gangrene of her left lower extremity requiring a midthigh amputation. The arteries from the amputated extremity revealed arteriosclerosis, medial degeneration and thrombosis. Arteriosclerotic lesions in the aorta and many other vessels also showed a striking similarity to human lesions.

MONDAY AFTERNOON

C. Bruce Taylor, Presiding

1:30

31. Anticoagulant Activity of Human Arterial Mucopolysaccharides

J. E. Kirk. From the Division of Gerontology, Washington University, St. Louis, Mo.

Extraction of acid mucopolysaccharide material was made from the intima-media layers of 27 samples of the descending thoracic aorta by the procedure of Dyrbaye and Kirk. The age of the subjects from whom the samples were obtained ranged from 3 to 76 years. Analysis of the isolated material showed the following average percentage composition: SO₄, 12.5; hexosamine, 24.2; uronic acid, 33.5. Eighty per cent of the hexosamine was galactosamine and 20 per cent glucosamine. No significant change with age in
the sulfate content of the samples was found. Analysis of the electrophoretically separated fractions failed to reveal the presence of \( \alpha \)-heparin in the material.

The anticoagulant activity of the mucopolysaccharide samples was determined by the procedure of Freeman, Engelberg, and Dudley. Each of the 27 samples was tested at 4 different levels by addition of 100, 200, 400, and 800 \( \mu \)g of the material, dissolved in 0.9 per cent NaCl solution, to aliquots of the plasma. For comparative purposes a coagulation time test with heparin sodium, U.S.P., added in quantities of 0.4, 0.8, 1.2, 1.6, 2.0, and 2.4 \( \mu \)g was run with each set of experiments.

The average coagulation times observed for 0, 100, 200, 400, and 800 \( \mu \)g of mucopolysaccharide material were, respectively, 5 ± 0.1, 11.8 ± 0.2, 16.1 ± 0.3, 23.3 ± 0.4, and 34.2 ± 0.8 minutes. These observations show that the arterial mucopolysaccharide material possesses a definite, but low anticoagulant activity. When compared on a weight by weight basis, the anticoagulant activity of the material was less than 1 per cent of that exhibited by heparin sodium (\( \alpha \)-heparin). The anticoagulant activity of the samples from children was moderately higher than the activity recorded for samples from adults.

The observed anticoagulant activity of human arterial mucopolysaccharides may constitute a factor of significance in connection with Duguid’s theory concerning the etiology of atherosclerosis.

1:45

32. Functional Activity of Arterial Mucopolysaccharides

Ira Gore and Bernard J. Larkey. From the Veterans Administration Hospital, West Roxbury, Mass. and the Department of Nutrition, Harvard School of Public Health and the Department of Pathology, Harvard Medical School, Boston.

It has been suggested that altered distribution of intimal acid mucopolysaccharides with atherosclerosis depicts an parallel alteration in the anticoagulant property of the intima which may underlie the occurrence of thrombosis as a complication of atherosclerosis. In pursuit of this thesis, human aortas were extracted for acid mucopolysaccharides using a modification of the techniques of Dyhrye and Kirk. Such extracts (MPS) proved to have anticoagulant properties similar to but less potent than those displayed by heparin. Neither substance displayed fibrinolytic activity when applied to clots of recalcified plasma. Since heparin also stimulates the formation of lipoid clearing factor when given intravenously, aortic MPS preparation was tested for and shown to have similar activity in white rats.

Chemically, the acid mucopolysaccharides isolated from beef aorta have been identified as chondroitin sulfates A and B, hyaluronic acid and heparitin sulfate. Chondroitin sulfate B has previously been shown to be an anticoagulant and except for quantitative differences displayed the same reactions as the aortic MPS when tested for anticoagulant properties, for lipid clearing factor stimulation, and for fibrinolytic effect. Hyaluronic acid and chondroitin sulfate A proved to be completely inert when tested by the same procedures. Although heparitin sulfate was not available for similar evaluation, the identity of the reactions of CSA-B and aortic MPS seems to suggest that the demonstrable anticoagulant lipid clearing stimulatory properties of the latter (MPS) are due to its content of the former (CSA-B).

2:00

33. Effect of Heparin on Serum Cholesterol and Triglycerides, Fecal Bile Acids, and Digitonin-Precipitable Sterols

H. Engelberg. From the Division of Laboratories, Cedars of Lebanon Hospital, Los Angeles, Calif.

The main channels of cholesterol excretion are via the fecal bile acids and fecal cholesterol (or its bacterial degradation product, coprosterol). Accordingly, these analyses were done, together with determinations of serum cholesterol and triglycerides, in human volunteers upon their normal diets before and after the administration of heparin (300-400 mg. daily in divided doses). The control and experimental periods of observation were 4-6 days each. It seemed valuable to determine the effect upon cholesterol excretion of enhanced triglyceride clearance from the bloodstream.

Daily bile acid excretion was increased during the period of heparin administration in all 4 subjects, the increase varying from 21-112 per cent. At the same time a decrease in fasting serum triglycerides and cholesterol occurred. The results demonstrate that cholesterol excretion is increased and serum cholesterol decreased, when serum triglyceride removal is facilitated by injected heparin. This is further evidence that one of the functions of circulating cholesterol is to aid in the transport of alimentary neutral fat, and that one of the causes of an increased cholesterol level is a protracted elevation of serum triglyceride. The results also indicate that an increase in bile acid excretion, such as occurs after the substitution of unsaturated for saturated fats.
in the diet, cannot necessarily be ascribed to an effect upon cholesterol excretion, per se, since it may also result from changes in a prior stage of fat metabolism.

2:15

34. In Vitro Effects of Heparin on Human Blood Lipoproteins

Daniel A. Sherber and Martin Marcus. From the Metabolic Research Laboratory, Fordham Hospital, New York, N.Y.

In studying the ease of extraction of lipoprotein cholesterol (REC), the observation was made that in vitro heparinized human plasmas (50 mg. per 100 ml. whole blood) usually demonstrated much higher values than untreated serum. At refrigerator temperature, approximately 27 per cent of the cholesterol is extracted by the alcohol-ether system after 20 hours compared to 6 per cent with no heparin added. Serum and other plasmas obtained by using other anticoagulants (sodium citrate, potassium oxalate, and double oxalate containing potassium and ammonium oxalates) all have equal REC values (6 per cent). Electrophoresis of the hydro-alcoholic residue showed that the REC method removes all the lipid from protein although only a small fraction diffuses into ether. The effect of heparin is to increase the rate and amount of diffusing lipid. This effect seems to depend on the ability of heparin to alter the gel-forming tendency of serum. Previous reported studies indicated a possible adrenal factor affecting lipid and protein binding. The relationship between this and the findings with heparin will be discussed.

2:30

35. Lipoprotein Lipase and Serum Lipids in Experimental Biliary Obstruction

Saul P. Baker, Piero P. Foda, Paul B. Szanto, and Alvin Dubin. From the Departments of Medicine, Physiology and Pharmacology, The Chicago Medical School, and the Department of Pathology and Hektoen Institute for Medical Research of the Cook County Hospital, Chicago, Ill.

An increased heparin-activated lipoprotein lipase response in Laennec's cirrhosis has been reported. A markedly decreased lipoprotein lipase response in obstructive jaundice was also reported. In order to more precisely evaluate lipoprotein lipase response in obstructive jaundice and correlate this response with serum lipids, the common bile duct was completely ligated in 7 normal dogs. Lipoprotein lipase response was determined and hepatic status and serum lipids were comprehensively evaluated on all dogs at weekly intervals after ligation. Control studies, including BSP excretion, were performed weekly on 6 of the dogs during a 3 week period prior to surgery.

A markedly decreased lipoprotein lipase response was observed in all 7 dogs after complete common bile duct ligation, thus confirming the decreased response in obstructive jaundice in man. Generally, lipoprotein lipase response was inversely proportional to total serum lipids, total serum cholesterol, esterified cholesterol, and lipid phosphorus levels. However, in 4 of the 7 dogs, sustained hyperlipemia (as high as 2,880 mg. per cent) and hypercholesterolemia (as high as 430 mg. per cent) were present for 2 to 5 weeks prior to the initial decrease in lipoprotein lipase. Hyperbilirubinemia, per se, did not appear to influence lipoprotein lipase response. In all 5 dogs that survived surgery by 4 weeks or more, a subsequent marked increase in lipoprotein lipase response was observed. This was generally, but not always, accompanied by a marked decrease in total lipids, total and esterified cholesterol, and lipid phosphorus. Postmortem microscopic examination of the livers of these animals revealed obstructive cholestasis and portal fibrosis.

It is suggested that the decreased lipoprotein lipase response in biliary obstruction is related to chronic cholestasis, and, indirectly, to serum lipid levels. The subsequent increase may be attributed to loss of hepatic parenchymal cells due to portal fibrosis.

2:45

36. Observations on Components of the Plasma Clearing System

J. L. Koppel, Lillian V. Novak, and John H. Olwin. From the Coagulation Laboratory, Department of Surgery, Presbyterian-St. Luke's Hospital, Chicago, Ill.

In vitro studies of human plasma clearing activity have been carried out in the presence and absence of purified human clearing factor preparations. The data obtained suggest that in only 25 per cent of all patients having little or no "inherent" plasma clearing activity is this deficiency due to a lack of the clearing enzyme proper. In the remaining 75 per cent it is caused by either the lack of a plasma cofactor or the presence of a clearing factor inhibitor or both. Although a distinct plasma cofactor appears essential for clearing as shown by the latter's disappearance as a result of ether treatment, no lack of this cofactor could be demonstrated in plas-
mas having deficient activity. However, these activities can be largely or completely restored by: (1) Adsorption of plasma with barium sulfate, (2) addition to plasma of citrate, oxalate, Na-verseenate, or certain sulphydryl inhibitors, and (4) heparin, polyethylene sulfonate or manuronate. The results suggest that, in these cases, the deficiency is due to excessive inhibitor levels and that the inhibitor may require free sulphydryl groups. Data obtained with a variety of other metal verseene salts indicate that calcium may also be essential for its action. It is of interest in this connection that of various purified coagulation factors studied only Ae-globulin (factor V) preparations strongly inhibit plasma clearing activity. Although some of the apparent characteristics of the clearing factor inhibitor are similar to those of Ae-globulin no evidence as to their possible identity has been obtained.

3:00

37. Quantitative Immunologic Differentiation of Human Serum β-Lipoprotein Fractions: Studies in Recent Myocardial Infarction

Saul P. Baker and A. S. Markowitz. From the Department of Medicine, The Chicago Medical School, and the Hektoen Institute for Medical Research of the Cook County Hospital, Chicago, Ill.

Production of antiserum to an ultracentrifugally-derived serum fraction from dogs on a thiouracil-cholesterol atherogenic dietary regimen has previously been reported. Subsequently, the serum fraction used as antigen was found to consist of a β-lipoprotein spectrum Sf 2-30 containing 3 peaks (Sf 3-7, Sf 8-11 and Sf 12-17) in dogs on atherogenic diets. In normal dogs, only one peak (Sf 3-7) was observed. Production of antisera to this Sf 2-30 spectrum, and its use in precipitin tests was subsequently reported. However, quantitative immunologic differentiation of β-lipoprotein fractions has not been accomplished heretofore.

Using pooled sera freshly obtained from patients with recent myocardial infarction, it has been possible to quantitatively extract Sf 10-100 (fraction I), Sf 5-15 (fraction II), and Sf 3-9 (fraction III) lipoprotein fractions in bulk employing a dextran sulfate complex and the ultracentrifuge. These fractions were then used as antigens in rabbits to produce antiseras. Specificity of each antiserum was established by differential absorption with the other 2 β-lipoprotein fractions. Quantitative determination of β-lipoprotein protein nitrogen for each fraction in whole sera tested was accomplished by micro-Kjeldahl analysis of precipitates.

Observations to date on a group of 44 patients with recent myocardial infarction yielded a mean of 1.44 mg. per cent protein nitrogen (range 0.93 to 1.70) for fraction I; a mean of 0.80 mg. per cent protein nitrogen (range 0.62 to 1.05) for fraction II; and a mean of 4.72 mg. per cent protein nitrogen (range 3.96 to 5.10) for fraction III. Six normal subjects yielded a mean of 0.62 mg. per cent protein nitrogen (range 0.49 to 0.72) for fraction I; a mean of 0.42 mg. per cent protein nitrogen (range 0.38 to 0.49) for fraction II; and a mean of 2.36 mg. per cent protein nitrogen (range 2.01 to 2.70) for fraction III.

It is suggested that quantitative immunologic studies of the protein moieties specific to fractions of ultracentrifugally-defined human serum β-lipoproteins may be useful in evaluating coronary atherosclerosis in man.

3:15

38. Immunochemical Reactions of Low Density Lipoproteins of Human Serum and Aortic Wall

Herbert J. Kayden, Beatrice C. Seegal, and Konrad C. Hsu. From the Department of Medicine, New York University College of Medicine, and the Department of Microbiology, Columbia University College of Physicians and Surgeons, New York, N. Y.

We have previously reported that saline extracts of homogenized portions of human aortic intima contain lipoproteins that can be fractionated by electrophoresis or ultracentrifugation. The phospholipid composition of β-lipoproteins (electrophoresis) and low-density lipoproteins (ultracentrifugation specific gravity < 1.063) of human sera have been compared with the phospholipid composition of comparable fractions isolated from aortic wall extracts. Although these lipoproteins are very similar in their physical properties, chemical analysis by silicic acid column chromatography and by differential alkaline hydrolysis, revealed striking differences in phospholipid composition.

Rabbits were immunized with the low-density lipoproteins derived from human sera and from saline extracts of human aortic wall. The reactions of these antisera with low density lipoproteins of serum and aorta were studied by means of the precipitin test, by double diffusion patterns in gels, and by immunoelctrophoresis. It was not possible to distinguish between the 2 antigens by any of the immunochemical procedures.
The two antisera were tagged with fluorescein and used in an attempt to identify the location of these specific lipoproteins in a segment of a human aorta removed during surgery for coarctation. No specific fluorescence could be detected in the aortic sections, either in the atheromatous plaque or in the vessel walls. The inability to determine reaction sites may be due to the marked autofluorescence of the aortic wall or to an absence of native lipoprotein in this preparation. Further studies using rhodamine-tagged antisera are being carried out.

3:30

39. Effect of Neomycin, Para-Aminosalicylic Acid and Other Antibacterial Drugs on the Serum Cholesterol Level of Man

Paul Samuel. From the Department of Medicine, New York University-Bellevue Medical Center, Post-Graduate Medical School, New York, N. Y.

It was reported that the oral administration of neomycin lowered the serum cholesterol level significantly in each of 10 patients. In the present study, neomycin (Mycefradin sulfate) was given orally to 18 patients at the daily dose of 1.5 to 2 Gm. for a period varying between 4 and 20 weeks. Serum cholesterol levels were lowered significantly in each patient by the average of 17 to 29 per cent during the administration of neomycin, the over-all average being 21 per cent.

Five patients were given 60 mg. of neomycin intramuscularly for a period of 3 weeks. This amount is equivalent to or higher than the proportion of oral dose (3 per cent) which is absorbed from the gastrointestinal tract. The serum cholesterol level of these patients failed to show appreciable changes.

Three patients were given 6 Gm. of PAS daily by mouth for 4 weeks without significant effect on serum cholesterol levels. Subsequently, 12 Gm. of PAS was given to 2 patients for 9 and 4 weeks respectively. The average serum cholesterol level was lowered by 24 per cent in one and 26 per cent in the other patient.

Phthalysulphathiazol, isoniazid, streptomycin, oxytetracyclin, polymyxin B sulfate, bacitracin, novobiocin and carbomyein were given each orally to 2 or more patients during a period of 2 to 4 weeks. No appreciable changes were noted in serum cholesterol levels following the administration of any of the above drugs.

The findings indicate that the effect of neomycin depends upon its action in the gastrointestinal tract. This might be due to the modification of the intestinal bacterial flora and/or the inhibition of intestinal enzyme systems. The mechanism of action of PAS cannot be explained at the present time.

3:45

40. Treatment of Obliterative Arterial Disease with Relaxin

Gus G. Casten and Hugh R. Gilmore, III. From the Research Department, Miami Heart Institute, and Department of Medicine, University of Miami School of Medicine, Miami, Fla.

Relaxin, a nonfeminizing hormone produced by the mammalian ovary, exerts a direct effect upon connective tissue. This material produces an increase in blood flow, evidenced by relief of Raynaud’s phenomenon and healing of ischemic ulceration in scleroderma, as previously reported by this laboratory. This report is a study of 12 patients with arteriosclerosis obliterans and 2 patients with Buerger’s disease treated with Relaxin during the past 2 years. All patients had advanced disease refractory to conventional therapy.

Relaxin intramuscular injections (20 mg. gelatine solution daily or 20 mg. repository solution twice weekly) were coupled with oral estrogen “priming” (Premarin 1.25 mg., 3 times weekly). Healing of ischemic ulceration has been demonstrated consistently. Clinical improvement in claudication and disappearance of rest pain has been apparent in all patients.

Surface skin temperature under conditions of maximal vasodilation was used as a measure of blood flow to the toes. The mean pretreatment skin temperature of 12 patients was 28.0 C. (normal range 31.0 ± 1 C.). Measurements at intervals of 1 month revealed a progressive rise with Relaxin therapy. After 3 months the mean level had increased to 31.0 C. (p > 0.001), and every patient studied showed an increase in temperature. This improvement is maintained in patients studied for over 1 year provided adequate dosage of Relaxin is given.

Relaxin presumably alters collagen and hence enhances perfusion from the arterial tree. Our experience to date indicates that this hormone will prove to be a significant advance in the therapy of oblitative arterial disease.

4:00

41. Serum Cholesterol Reduction with D-Thyroxine

Richard J. Jones. From the Department of Medicine, University of Chicago, Chicago, Ill.

Manifestly pure d-thyroxine was administered to 15 patients with clinically stable angina pectoris or a healed infarct who were euthyroid but hy-
percholesteremic. During a 6 month control period, 10 had mean serum cholesterol levels above 300 mg. per cent and the remainder above 250 mg. per cent. Oxygen consumption, pulse rate, blood pressure, and serum lipids were determined in the basal state before and after 3 months on d-thyroxine, and the serum cholesterol was followed at biweekly intervals throughout. Most patients were started on 8 mg. per day, initially, but later this was adjusted downward in some patients to 6 or 4 mg. per day.

Each of the patients showed a dramatic fall in serum cholesterol within 2 weeks, which was sustained and showed a tendency to “escape” toward control levels in only 2 cases. The mean reduction from control levels was 25 per cent. At the dosages employed, d-thyroxine was not completely free of calorigenic effects. Certain patients had an increase in BMR, lid lag, tremor, or frequency of angina during the treatment period. These effects, plus our experience in titrating d-thyroxine and l-thyroxine in 1 myxedema patient, indicate that, in large enough dose, d-thyroxine certainly does have a calorigenic effect in humans. On the other hand, a striking hypcholesteremic effect is seen, frequently with doses of only 4 to 6 mg. per day, which has been sustained for at least 3 months. With proper individualization of dose, this hypcholesteremic effect may be achieved short of undesirable side effects.

4:15

42. Effect of Nonionic Detergents on Electrophoretic Mobility and Ultracentrifugal Distribution of Human Serum Lipoproteins


The admixture of 0.5-1.5 per cent Tween in the buffer used for paper electrophoresis produced marked changes in the electrophoretic mobility of serum lipoproteins, so that the a1-lipoproteins no longer migrated with the a1-globulin but in a position close to the origin and behind the β-lipoproteins. The same changes were produced by incubating 0.5 per cent Tween with serum at 37°C for 1-2 hours. The identity of the altered lipoproteins bands was established by the use of isolated a1- and β-lipoproteins. These changes were apparently produced by the strong attachment of Tween on the lipoprotein molecules as shown by studies using 131I-labeled Tween 80 and could not be reversed by prolonged dialysis.

Changes were also evident in the flotation patterns of lipoproteins. Serum incubated with 0.5 per cent Tween 40 for 1 hour and centrifuged in a preparative ultracentrifuge for 24 hours at 114,000 G in a medium of 1.063 density, showed 80-90 per cent of the lipoproteins accumulated in the top fraction as determined by lipid analysis. Electrophoretically such fractions contained both the a1- and β-lipoproteins. With ultracentrifugation at densities of 1.018 and 1.006 a decrease in the amount of lipoproteins accumulated at the top fraction was observed, as compared with lipoprotein fractions of the same density but without Tween.

Studies are in progress using the analytical ultracentrifuge to determine detailed patterns of the alterations of the lipoprotein molecules as well as the in vivo effect of these detergents on the lipoprotein patterns of experimental animals.

TO BE READ BY TITLE

43. Cholesterol Depressing Effect of Fruits, Vegetables and Legumes when Substituted for Sucrose and Skim Milk in Human Diets

Joseph T. Anderson, Francisco Grande, and Ancel Keys. From the Laboratory of Physiological Hygiene, University of Minnesota, and Hastings State Hospital, Hastings, Minn.

Comparisons between populations subsisting on different diets suggests that not all of the mean differences in serum cholesterol values are accounted for by dietary fats. Accordingly, controlled experiments were carried out on men with diets differing in the proportions of carbohydrate calories supplied from various food sources. Diets IL and AL provided 13 per cent of calories from proteins and 16 per cent from fats and were identical except for 18 per cent of total calories from sucrose and milk carbohydrate in AL exchanged for equal calories in carbohydrates in fresh fruit, vegetables and legumes. Seven men subsisted on diet AL, then changed to diet IL for 6 weeks each, while 7 matched men made the reverse change. Similarly, 2 matched groups of 7 men each subsisted in crossover experiments on diets IM and AM which corresponded to IL and AL except that total fats provided 31 per cent of calories.

Serum cholesterol averages were 16 mg. per cent lower on the IL than on the AL diet and 19 mg. per cent lower on the IM than on the AM diet and these differences were highly significant statistically. Gas chromatograph analysis of the extracted mixed fats from the diets as fed indicated equally of IL vs. AL and of IM vs. AM diet in polyunsaturated fats but the IL and
IM diets provided a slightly higher (+3 per cent and +1 per cent) percentage of calories from saturated fatty acids than in the comparison AL and AM diets. These fat differences would, of themselves, produce slight serum cholesterol differences in the opposite direction to those observed. It is concluded that sucrose and milk sugar tend to produce higher serum cholesterol values than equal calories of carbohydrates contained in fruits, vegetables and legumes.

44. Coronary Blood Flow and Metabolic Factors in Experimental Atherosclerosis

T. A. Balourdas, J. J. Spitzer, M. N. Croll, and J. C. Scott. From the Institute of Cardiovascular Research and Department of Physiology, Hahnemann Medical College and Hospital of Philadelphia, Pa.

Experimental Atherogenesis was induced in mongrel dogs by administration of I\(^{131}\) followed by a high cholesterol diet.

The hypothyroidism induced by radioactive iodine (30-35 μc) was shown by a marked reduction in BMR, low PBI conversion ratio and elevated blood cholesterol level. The cholesterol diet consisted of 2.5 per cent cholesterol regime given daily for 8 to 14 months.

Fifteen experiments were performed using the nitrous oxide desaturation method for determination of coronary blood flow. Observations were made on the same dogs under normal conditions, after the development of hypothyroidism and again after 8 to 14 months of cholesterol diet. Coronary sinus and femoral artery blood samples were analyzed for O\(_2\), CO\(_2\), N\(_2\)O and unesterified fatty acids (UFA).

In normal dogs average values were similar to those previously reported. The hypothyroid condition showed 25-30 per cent reduction in: coronary sinus flow, LV O\(_2\) concentration, heart rate, cardiac index and LV work, but increased CVR. Atropine increased: CSBF, LV O\(_2\) concentration, mean arterial blood pressure, heart rate, cardiac index, LV work, and decreased LV efficiency and CVR.

UFA uptake by the myocardium was significantly higher in normal than in the hypothyroid. Atropine increased UFA uptake in both groups.

In the atherosclerotic dogs the CSBF, CVR, LV efficiency, LV O\(_2\) consumption, UFA, and other factors were lower than in hypothyroid condition. Hypercholesterolemia was increased. After each terminal experiment postmortem findings showed generalized atherosclerosis of coronary and systemic arteries confirmed microscopically.

45. Effect of an Anion Exchange Resin on Serum Cholesterol in Man


Tennent et al. have described lowering of serum cholesterol in animals fed the chloride salt of a basic anion exchange resin. This material (MK-135) is presumed to exert its effect by sequestering bile acids.

MK-135 was administered to 26 patients for periods ranging from 2 to 34 weeks. The patients were divided into 4 clinical groups: coronary heart disease, familial hypercholesteremia, mild diabetes mellitus, and normocholesteremia. The preparation was given in 4 equal doses totalling 15 Gm. per day taken with each meal and at bedtime. Six of the subjects did not display appreciable cholesterol lowering until this dose was doubled. Serum total cholesterol and cholesterol ester concentrations were determined at frequent intervals by the Sperry-Schoenheimer method.

Serum total cholesterol levels were lowered by more than 10 per cent in 23 of the 26 patients. Cholesterol ester changes paralleled changes in total cholesterol. The average decrease in serum total cholesterol for all the subjects during treatment was 20 per cent (p < 0.001).

No toxic side effects were seen, however 6 patients developed some constipation while on the drug. One patient had nausea and vomiting and, with this exception, there was no weight loss or evidence of impaired digestion in any of the subjects. Six subjects who were studied for 6 weeks or longer maintained lowered values of serum total cholesterol without evidence of escape from the medication. Of the 15 patients whose cholesterol levels were followed after termination of MK-135 treatment, 8 showed a rebound to levels ranging from 4.6 to 30.6 per cent above control values. After approximately 2 weeks this "rebound" group returned to control levels.

The results indicate that, within limits, a direct relationship existed between amount of resin ingested and degree of depression of cholesterol levels.

46. Thyroid Hormones and Cholesterol Metabolism: Effects of Side-Chain Substitutions

Maurice M. Best and Charles H. Duncan. From the Department of Medicine, University of Louisville School of Medicine, Louisville, Ky.
The addition of 1 per cent cholesterol and 0.5 per cent thiouracil to the diet of the albino rat for 2 weeks resulted in an increase in liver cholesterol from 211 to 1,336 mg. per 100 Gm. and an increase in thyroid weight from 5.4 to 18.1 mg. per 100 Gm. body weight. We have previously reported (1956) that, as compared to l-thyroxin, its formic acid analog (tetraiodothyroformic acid) is more active in preventing this increase in liver cholesterol than in inhibiting the thiouracil-induced goiter.

In the present study the effects of other modifications of the side-chain of l-thyroxin and l-triiodothyronine have been similarly studied. Approximately 300 Gm. rats were employed and each analog was administered subcutaneously for 2 weeks in an amount estimated to give 80 per cent goiter inhibition.

The daily administration of .004 μM per 100 Gm. of l-thyroxin resulted in 94 per cent inhibition of goiter and mean liver cholesterol of 774 mg. per 100 Gm. Daily dose per 100 Gm., mean goiter inhibition and mean liver cholesterol resulting from administration of compounds in which the following substitutions for the l-alanine side-chain of l-thyroxin had been made were: d-alanine, .02 μM, 80 per cent, 586 mg. per 100 Gm.; formic acid, 4.0 μM, 73 per cent, 402 mg. per 100 Gm.; propionic acid, .02 μM, 100+ per cent, 732 mg. per 100 Gm.; and butyric acid, .04 μM, 100+ per cent, 862 mg. per 100 Gm.

Daily administration of .001 μM per 100 Gm. of l-triiodothyronine resulted in 100+ per cent inhibition of goiter and mean liver cholesterol of 719 mg. per 100 Gm. Compounds in which substitutions for the side-chain of l-triiodothyronine had been made gave the following results: d-alanine, .016 μM, 93 per cent, 449 mg. per 100 Gm.; formic acid, 1.32 μM, 80 per cent, 615 mg. per 100 Gm.; acetic acid, .01 μM, 50 per cent, 920 mg. per 100 Gm.; and propionic acid, .004 μM, 69 per cent, 910 mg. per 100 Gm.

Although failure to obtain identical degrees of goiter inhibition precludes exact comparison, it would appear that d-triiodothyronine and d-thyroxin possess to some extent the disproportionately greater effect on cholesterol metabolism displayed by tetraiodothyroformic acid.

47. Inhibitory Action of Dietary Factors on Experimental Atherosclerosis in Young Cockerels

Clyde T. Caldwell. From the Department of Nutrition and Metabolic Diseases, The Upjohn Company, Kalamazoo, Mich.

The protective action of dietary factors for young cockerels on atherogenic regimen has been studied. As much as 98 per cent decrease in plaque formation and 54 per cent reduction in serum cholesterol may result from treatment with a combination of certain materials.

Studies of the effect of dietary fat, protein, carbohydrate, and certain vitamins and minerals on the atherosclerotic process emphasize the interrelatedness of metabolic reactions involved. They suggest the probable value of providing in the diet for more adequate enzymatic control under atherosclerotic conditions. They indicate the importance of studying vitamins and hydrolytic products of nucleic acids, as constituents of enzyme systems, in larger than normal dosage levels.

The response of young cockerels to various mixtures of these materials has been studied in both cholesterol- and estrogen-induced atherosclerosis. The amount of each substance in the mixtures was based upon an indicated number of times the normal requirement, and in most instances that amount was held constant during the experimental period.

The atherogenic diet used in the 8 weeks cholesterol method contained 0.5 per cent cholesterol incorporated into a normal chick growing mash. Two and one-half milligrams of estradiol cyclopentylpropionate in 0.5 ml. cottonseed oil was administered intramuscularly at the beginning of the 1-week experimental period as the only atherogenic agent for the estrogen method. Results obtained by each method in the study of various combinations of these materials compare favorably. The antitherogenic response was more pronounced for certain mixtures than for individual substances.

The data to be presented support the concept of a high degree of interrelatedness among metabolic reactions involved in atherosclerosis, and that certain dietary factors in pharmacologic amounts are able to decrease greatly the severity of the disease process in cockerels.

48. Enhanced Blood Coagulation Effects of Soaps from Hydrogenated Food Fats

Herbert L. Davis and Nora L. Davis. From the Departments of Biochemistry and of Surgery, University of Nebraska College of Medicine, Omaha, Neb.

The consumption of fats rich in saturated acids appears to be especially hazardous in terms of correlation with arteriosclerotic lesions and cardiovascular deaths. Saturated fatty acids tend to be about twice as effective in raising plasma cholesterol concentrations as equal weights of the polyunsaturated acids are in lowering them. Previous experiments showed that the ability to accelerate recalcification gel rates of citrated human plasmas rises sharply with increased chain lengths of the
saturated fatty acid soaps, and is markedly lower for comparable soaps of unsaturated fatty acids. The coagulation index (maximum per cent lowering of gel time/millimols of soap per liter required to give this value) rises from 1 for C₆ (caprylate) to 160 for C₁₈ (stearate). In a similar series of plasmas, soaps of oleic, linoleic, and linolenic acids all gave C.I. values about 8, which is the value of C₁₂ (laurate). Tests on many plasmas have shown that sodium stearate is from 6 to 20 times as coagulant as sodium oleate.

Now corn oil, cottonseed oil, and soy bean oil have been hydrogenated to varying lower iodine values, and their fatty acids added as sodium soaps to human plasmas before recalcification. With cottonseed and soy oils, hydrogenation to the commercial I.V. 80 value approximately doubles the coagulation index. Corn oil is less affected by such a degree of hydrogenation. All 3 oils on further saturation showed continued elevation of C.I. values.

Such findings suggest that diets rich in saturated fats or in hydrogenated fats may be more likely to produce enhanced coagulability of the blood, than would similar diets based on natural oils rich in oleic acid and the polysaturated fatty acids. This should be a major factor in stress episodes, tending to produce thromboembolic phenomena and consequent coronary hazards.

49. Persistent Experimental Aortic Aneurysms in Dogs

Steven G. Economou, George E. Cox, and C. Bruce Taylor. From the Departments of Surgery and Pathology, Presbyterian-St. Luke's Hospital, Chicago, Ill.

Acetrizoate (Urokon) in a 70 per cent solution was injected intramurally into the thoracic aortas of mongrel dogs which were sacrificed and studied at intervals of from 3 to 30 weeks. Gross aneurysms developed and persisted. The lack of repair was attributed to the absence of injury and stimulation of subendothelial layers since nearly all repair is achieved by proliferating subendothelial cells. To further test this thesis aneurysms were produced by dissection of 60 to 70 per cent of the outer media without injuring the inner media and subendothelial layer. Aneurysms developed immediately and persisted up to 30 weeks, the longest period of observation. Other forms of arterial injury which resulted in injury and stimulation of the subendothelial layers invariably resulted in formation of thick intimal sears. In another series of animals, one-half of each aneurysm was frozen in situ so as to injure the subendothelial layer. Profuse subendothelial sears developed at the site of the transmural freezing; the adjacent area, where no subendothelial injury had occurred, showed no evidence of repair. These findings demonstrate that the stimulus to repair of aneurysms, due to loss of medial structure, was trauma to the subendothelial layer.

50. A Physical Method for Grading Fatty Streaks in the Aorta

Douglas A. Eggen and Russell L. Holman. From the Department of Pathology, Louisiana State University School of Medicine, New Orleans, New Orleans, La.

The early lesions of human atherosclerosis, fatty streaks, have been quantitated by a photographic-photometric method applied to the intimal surface of aortas that have been stained with Sudan IV. With the aid of appropriate filters, 2 very high contrast (Kodak) negatives are made for each aorta preparation. One negative is transparent only in those areas which are stained by Sudan IV and the other negative is transparent over the whole aorta image. A photometric measurement of the mean densities of these 2 negatives then gives a measure of the fraction of the area occupied by fatty streak.

The method is time-consuming and somewhat difficult to control because of variations in background staining. It is not recommended for the grading of large numbers of preparations but is useful in research studies. Measurements made by this method on 2 sets of human aorta which had previously been graded visually gave correlations of 0.97 and 0.92 with these visual estimates.

51. Liver Function Studies in Humans with Elevated Levels of Serum Cholesterol and/or Low-Density Lipoproteins

H. Engelberg. From the Division of Laboratories, Cedars of Lebanon Hospital, Los Angeles, Calif.

The liver is fundamentally involved in various phases of fat and cholesterol metabolism. Kupffer cell phagocytosis of chylomiera has also been proposed as a possible physiologic pathway for the removal of alimentary neutral fat from the bloodstream. Accordingly, a battery of liver function tests were done in about 50 private patients without evident liver disease who had markedly elevated levels of serum cholesterol and/or low-density lipoproteins.

In approximately 10-15 per cent of the subjects there was a slight abnormality of the BSP test, varying from 8-12 per cent (normal less than 7 per cent). Evidence will be presented which sug-
suggests that in some cases this finding may be secondary to the elevated lipoprotein levels. In 85-90 per cent of the group all liver function tests were normal. Thus, in the majority of individuals with hypercholesterolemia and hyperlipoproteinemia, no disturbance of liver function can be demonstrated with presently available techniques.

52. Effect of Nicotinic Acid on Conjugation Pattern of Bile Acids in Man

Robert B. Failey, Jr., Earlene Brown, and M. E. Hodes. From the Indiana University School of Medicine, Indianapolis, Ind.

The oxidation of cholesterol to bile acids has been shown to be an important step in cholesterol excretion. Nicotinic acid in high dosage is effective in lowering serum cholesterol levels in man. The present report deals with work undertaken to establish a relationship between administration of nicotinic acid and bile acid excretion.

Bile was obtained from 3 patients by duodenal intubation, first while they were taking nicotinic acid in divided dosage at 2 Gm. a day, subsequently 1 to 3 days following cessation of medication. Bile acids as taurine and glycine conjugates were separated by column chromatography and individual acids determined colorimetrically and spectrophotometrically.

Following cessation of medication taurocholic/glycocholic acid ratio fell 47 and 93 per cent in 2 patients, rose 12 per cent in 1. Taurochenodeoxycholic/glycochenodeoxycholic ratio fell 52, 57, and 93 per cent. Taurodeoxycholic/glycodeoxycholic ratio fell 100 per cent in 1 patient, could not be determined in 2. No consistent reaction pattern could be shown in relationships of the individual unconjugated acids.

Conjugation of glycine with nicotinic acid and its excretion as nicotinuric acid is a probable explanation for these observations. Since animals conjugating bile acids predominantly with taurine are in general more resistant to atherosclerosis than are those conjugating with glycine, taurine conjugation induced by nicotinic acid may represent a more efficient means of cholesterol excretion.

53. Effect of Triiodothyropropionic Acid on Blood Lipids

Paul F. Flynn, Stanford Splitter, Harry Balch, and Laurance W. Kinsell. From the Institute for Metabolic Research, Highland-Alameda County Hospital, Oakland, Calif.

The effect of triiodothyropropionic acid on the blood lipid levels was studied in patients with different degrees of hyperlipemia. Causes of the elevated blood lipid values varied. Bloods were at first drawn weekly, then bimonthly. The following plasma analyses were made: Cholesterol total and esters, total phospholipids, unesterified fatty acids, "freely extractable lipids," and plasma total lipids.

A number of lipid baseline values were obtained for each patient. Diets and other medication had been stable for a considerable period prior to the use of this agent.

The study was designed to determine: 1. The lipid-lowering effects of triiodothyropropionic acid on a group of 15 patients. 2. The effects of the substitution of placebos for this agent on the same group of subjects. 3. The advantages, if any of triiodothyropropionic acid over the use of dessicated thyroid as a means of lowering lipid levels.

A majority of subjects showed a highly significant drop in plasma total lipids, ester and total cholesterol, and freely extractable lipids when the agent was administered. Uniformly, phospholipids fell. Unesterified fatty acids showed a significant rise in most subjects. The placebo study is currently under way.

Over 50 per cent of the subjects at some time during the study had a pulse rate of over 110. This finding correlated with the size of the dose in the individual, but not in the group as a whole. Many of the clinical effects of dessicated thyroid were observed, and in those subjects who had radioactive iodine uptake studies, suppression of thyroid activity was found to be present. It is believed that the rise in unesterified fatty acids seen in most of these subjects may be similar to the rise in unesterified fatty acid levels seen in clinical thyrotoxicosis.

In addition to the foregoing, the comparative effects of physiologic or pharmacologic dosage of dessicated thyroid in the same patients will be reported. At the time of submission of this abstract it is not clear whether triiodothyropropionic acid has any advantage over dessicated thyroid.

54. Tissue Steam-Volatile Fatty Acids in the Albino Rat

William C. Foster. From the Laboratory of Physiology Research, Misericordia Hospital, Philadelphia, Pa.

A steam distillation apparatus has been modified for the determination of steam-volatile fatty acids (SVFA). SVFA were distilled from blood, kidney, liver, salivary glands, stomach, duodenum, skeletal muscle, and spleen of Wistar-strain albino rats, weighing between 500 and 600 Gm. and approximately 1 year of age. The animals were maintained on the Dietrich and Gambrill laboratory diet for rats which contained 5 per cent fat, ad
libitum. Approximately 1 Gm. of tissue was homogenized in a tissue grinder in 2 per cent zine sulfate, followed by 0.5 N sodium hydroxide. The SVFA were then determined by a modification of the method of McClendon. The average rat blood SVFA compared favorably with human blood results of 0.0035 in the former and 0.003 N in the latter. The blood-hear muscle ratio was found to be approximately 1:4, the blood-kidney ratio was of the order of 1:6, and a high ratio of 1:8 was that of blood-liver.

55. Distribution of Atheromatous Plaques in the Aorta, Renal and Coronary Arteries

Seymour Glagov and Donald A. Rosove. From the Department of Pathology, University of Chicago, Chicago, Ill.

Regardless of etiologic factors in genesis of atherosclerosis, hemodynamic factors may affect the localization of atheromatous plaques. Studies on hemodynamic factors have usually been limited to investigation of local conditions at ostia or bifurcations, or to peculiarities of vessel contour, etc. However, hemodynamic factors may also vary in major arteries because of differences in blood flow in the tissues supplied. Because of known and presumed differences in blood flow in the myocardium and kidney, the distribution of atherosclerotic plaques was compared in the coronary and renal arteries as well as in the aorta.

The aorta, main coronary and renal arteries obtained from 200 consecutive autopsies (106 males, 94 females, ages 5 to 83) were fixed and preserved in plastic bags. The thoracic aorta, abdominal aorta, coronary and renal arteries, ranked according to the extent of atherosclerosis, were graded 0 to 4+.

In the entire series coronary atherosclerosis was 1+ or greater in 140; coronary artery atherosclerosis was greater than renal artery atherosclerosis in 137 and equivalent in 3. In no instance was renal atherosclerosis greater than coronary atherosclerosis. The findings establish beyond doubt that the renal arteries are spared or relatively spared of atherosclerosis; this is in spite of the fact that the renal arteries originate from a segment of the aorta which often has severe atherosclerosis. A pattern of distribution, coronary->>abdominal aorta->renal aorta was present in specimens from 14/20 patients with diabetes, 44/60 patients with hypertension, and 7/8 patients who died of myocardial infarction. It will be shown that this pattern of distribution is also seen in many vascular trees from patients without diabetes, hypertension or myocardial infarction. Hemodynamic factors which may in part account for the selective localization of atherosclerotic plaques in these arteries will be discussed.

56. Effect of Dietary Fat and Ethanol on Serum Cholesterol Concentration

Francisco Grande, H. W. Heupel, D. S. Amatuzio, and L. J. Hay. From the Jay Phillips Research Laboratory of Mount Sinai Hospital and The University of Minnesota, Minneapolis, Minn.

Total serum cholesterol concentration was measured in groups of apparently healthy dogs subsisting on diets of different fat content, with and without administration of ethyl alcohol. A reversal, or switchback, design was adopted in all the experiments. The effect of increasing the fat content of the diet from 4 per cent of the total calories to 40 per cent (from lard) was tested in 18 dogs. The mean serum cholesterol concentration on the low fat diet was 135 ± 6.0 mg. per 100 ml., as compared with 188 ± 7.5 mg. per 100 ml. on the high fat diet. The mean difference (53 ± 7.2 mg. per 100 ml.) is highly significant (p = 0.0001).

Administration by stomach tube of 1.65 Gm. of ethanol per Kg. per day, resulted after 2 weeks in a mean increase of serum cholesterol of 52 ± 8.2 mg. per 100 ml., in 8 dogs subsisting on the low fat diet (p < 0.001). The same dose of alcohol given to 8 dogs subsisting on the high fat diet (40 per cent of total calories from lard) produced a mean increase of serum cholesterol concentration after 2 weeks of 83 ± 11.2 mg. per 100 ml. (p = 0.0002).

Similar results were obtained when the same amount of alcohol was given to dogs subsisting on a high fat diet (40 per cent of total calories from fat) in which sunflower oil was used in place of lard.

The serum cholesterol concentration decreased when alcohol administration was discontinued, reaching the pre-alcohol level in about 2 weeks.

Administration of 3 oz. of whiskey per day (0.45 Gm. of ethanol per Kg. per day), to 60 healthy men subsisting on a high fat diet (40 per cent of total calories from fat, approximately) for 3 weeks, failed to produce any significant change in serum cholesterol concentration.

Results of experiments in man with higher doses of alcohol (1.36 Gm. of ethanol per Kg.) will be reported.

57. Studies on Protein Component of β-Lipoprotein

Scott Grundy, A. Clark Griffin, and Harold L. Dobson. From the Departments of Medicine and Biochemistry, Baylor University College of Medicine, Houston, Tex.
Despite extensive research on the properties of serum lipoproteins, relatively little is known regarding the protein portion of these complexes. The protein moiety may be studied while combined with lipid or following removal of the lipid. From lipoproteins of various classes we have removed the lipid by extraction and studied the resulting protein portion. It would appear that the protein moiety of the very low-density lipoproteins differs from that of the medium-density and high-density lipoproteins. This would suggest that if low-density lipoproteins are converted to higher density ones a change in the protein moiety must also occur. This ability to convert very low-density lipoproteins to higher ones appears to be absent in some patients with essential hyperlipemia, and this may represent a protein defect as well as a lipid defect. Rabbits fed cholesterol appear to have the same deficiency in conversion of lipoproteins to a higher density. It has also been observed that these lipoproteins have the ability to bind further lipid substances such as cholesterol and lipid containing surface active agents.

58. Nephrosclerosis and Hypertension in the Absence of the Adrenal Gland

Dwight J. Ingle and George F. Wilgram. From the Ben May Laboratory for Cancer Research, University of Chicago, Chicago, Ill.

While the etiology of essential hypertension is obscure it has been claimed that derailment of adrenal cortical function is a principal causative factor. The supporting evidence comes from studies in which salt loaded uninephrectomized rats were overdosed with adrenal cortical hormones. In extension of an earlier study we have kept 14 male and 23 female adrenalectomized, uninephrectomized rats either on a 4 or 12 per cent salt diet without cortical hormone therapy for 6 months. Almost all experimental animals showed some degree of damaged convoluted tubules with cast-formation and early involvement of the glomeruli. Average blood-pressure rose significantly above normal. In 6 animals incipient nephrosclerosis was definitely present despite the absence of the adrenal gland. These findings are interpreted as providing preliminary evidence that high salt loads alone are capable of inducing the above mentioned pathology and that the adrenal hormones play but a permissive role in the induction and in the perpetuation of nephrosclerosis and hypertension.

59. Serum Cholesterol in Man and Complex Carbohydrates in Diet

Ancel Keys, F. Grande, and J. T. Anderson. From the Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis, Minn., and the Hastings State Hospital, Hastings, Minn.

Populations characterized by low serum cholesterol values generally subsist on diets low in saturated fats but frequently high in vegetables and fruits that conceivably could influence the serum cholesterol. Such diets are relatively high in complex carbohydrates, particularly fiber, and pectin. To study these questions, 4 controlled experiments were made on groups of men subsisting on constant diets except for being with or without added (15 Gm. daily) cellulose fiber ("alphaeal") or U.S.P. pectin for alternating 3-week periods. In each experiment, after 4 weeks of stabilization control, half the men received the supplement in the first period but not in the second while the other men (group-matched) followed the reverse order. Fourteen men on a controlled diet of the usual American type showed no effect on serum cholesterol of adding or removing fiber. Another 14 men on another diet with the same amount and kind of fat and equal content of protein and carbohydrate likewise showed no change of serum cholesterol when given fiber. Twenty-six men on a diet similar to the usual American diet but made high in total protein for another purpose showed an average fall of serum cholesterol of 9.3 mg. per cent (p = 0.02) when receiving pectin. On an equivalent modified form of this diet, the 26 men showed serum cholesterol averaging 10.0 mg. per cent lower when receiving pectin than without this supplement (p < 0.001). It is concluded that 15 Gm. of pectin has a significant cholesterol lowering effect which on these diets amounted to 5 per cent of the pre-existing level.

60. Diurnal Serum Lipid Levels of Children with Cystic Fibrosis of the Pancreas

Peter T. Kuo and Nancy N. Huang. From the Hospital of the University of Pennsylvania, Philadelphia, Pa.

A decreased fat absorption has often been demonstrated in children with cystic fibrosis of the pancreas. This naturally imposed fat restriction, which is controllable to a certain extent by pancreatic extract, offers a unique condition for the study of the effect of dietary fat upon the serum lipid concentrations. Diurnal serum lipid variations of 9 children with cystic fibrosis of the pancreas and 7 normal controls on mixed diets supplemented with predigested proteins and carbohydrates were studied before and after the use of pancreatic extract. During each study, serum triglycerides, phospholipid and cholesterol were determined at 4 hour intervals for 24 hours.
In none of the children were any consistent diurnal serum cholesterol and phospholipid patterns observed. In both the normal and diseased groups the mean serum cholesterol concentrations were 175 and 124 mg. per cent and the phospholipid 180 and 165 mg. per cent respectively. In all 9 patients with pancreatic disease diurnal serum triglyceride curves remained flat following meals. These curves dipped slightly after midnight and rose to small peaks with prolonged fasting. Following the use of pancreatic extract, the serum triglyceride curves of 6 out of the 9 patients changed toward normal, showing significant postprandial rises and sharp dips to the respective fasting levels before breakfast. Pancreatic extract administration did not alter the serum triglyceride curves of the normal controls. Despite the chronic decrease in fat absorption, the mean fasting serum triglyceride concentrations of the patients was the same as that of the normal children.

61. Relationship between Changes in Coagulation Indices and Blood Lipids during Alimentary Lipemia

A. Little, E. A. Murphy, J. F. Mustard, and H. M. Shanoff, From the Department of Veterans Affairs, Sunnybrook Hospital, Toronto, and Department of Medicine, University of Toronto, Toronto, Canada.

Following fat ingestion, there is an increase in serum lipids and acceleration of clotting. Phospholipids and triglycerides appear to influence clotting. Therefore, the changes in these lipids and the coagulation indices were studied during alimentary lipemia in humans.

Forty-five fasting males ages 30-50 (19 coronary patients and 26 controls) were fed a breakfast rich in dairy fats and eggs. Blood samples were obtained before and 5 hours post cibus.

During alimentary lipemia, there was a significant change in serum triglyceride, phospholipid, Russell viper venom time and plasma Christmas factor activity in both groups. The whole blood clotting time (in silicone tubes) decreased significantly in the coronary group only. The serum total and free cholesterol showed little change. Significant correlations between the serum lipid changes and the changes in the coagulation indices were found only in the coronary or the combined groups and not in the control subjects.

Serum triglyceride correlated significantly with plasma Christmas factor activity changes in the combined group (r = .44, p < .5), while in the coronary group, the significance was borderline. Triglyceride correlated with the whole blood clotting time in the combined and coronary groups (r = .46 and .54, respectively). There was no correlation of triglyceride with Russell viper venom time.

Serum phospholipid correlated with plasma Christmas factor activity changes and only in the coronary group (r = .47).

It is concluded that changes in plasma Christmas factor activity may be related to changes in serum triglyceride and phospholipid following a fat meal in susceptible subjects. Changes in whole blood clotting time appear to be related to changes in triglycerides alone. Russell viper venom time appears to be unrelated to changes in serum lipids. This suggests that the various clotting tests may be influenced by different lipid fractions during alimentary lipemia.

62. Comparative Effects of Glycoamyline and Nicotinic Acid on Experimental Cholesterol Atherosclerosis in Rabbits

Joseph M. Merrill, Jean Burkhalter, Bonnie Keith and Walter Earley. From the V.A. Hospital, Nashville, Tenn.

The major portion of administered nicotinic acid is excreted as a methylated derivative and glycocyamine is methylated to creatine. These excretory phenomena offered the possibility of investigating the comparative effects of these 2 substances on experimental cholesterol atherosclerosis in rabbits.

In this study groups of rabbits (6 adult males per group) were fed a stock diet plus 2 per cent cholesterol; a stock diet plus 2 per cent cholesterol with 0.4 per cent glycocyamine and a stock diet plus 2 per cent cholesterol with 0.4 per cent nicotinic acid. Serum cholesterol and body weight were determined at weekly intervals. After 8 weeks of the experimental diets the animals were killed and total cholesterol of the aorta and liver of the rabbits measured.

Analysis of the data obtained revealed that the average weekly serum cholesterol was lowest in the group of animals fed the nicotinic acid. After the third week of the experimental diets, the animals fed the glycocyamine had lower serum cholesterol than did the animals fed cholesterol alone. The differences, however, are not statistically significant. The aortic and liver tissue cholesterol paralleled the changes in serum cholesterol.

Measurement of the effect of short term feeding of glycocyamine on the incorporation of sodium acetate 1-C14 into liver cholesterol of rats indicates that the addition of the glycocyamine to the diet of the rat is associated with a twofold increase in incorporation of labeled carbon into liver cholesterol.
The results of this study suggest that niacinic acid was more effective in preventing the anticipated rise in serum and aortic tissue cholesterol than was a similar amount of glycoceamine.

63. Hormonal Factors in the Partition of Lipoprotein Cholesterol in Healthy Subjects

Campbell Moses and T. S. Danowski, From the Addison H. Gibson Laboratory and the Renziehausen Department of Research Medicine of the University of Pittsburgh School of Medicine, Pittsburgh, Pa.

After control variations in the partition of cholesterol, lipid phosphorus and triglyceride in the serum α- and β-lipoprotein fractions were determined in 84 healthy, institutionalized men, varying doses of desiccated thyroid, triiodothyronine, triiodothyroproprionic acid, and tetraiodothyroproprionic acid were given for periods up to 18 months. Twelve subjects receiving 2 mg. of triiodothyronine for 99 days had a fall in mean total cholesterol from 225 to 200 mg. per cent. This decrease was maintained and was largely due to a fall in the β-lipoprotein fraction. A similarly persistent but even greater fall in β-lipoprotein cholesterol was noted with 4 mg. of triiodothyroproprionic acid. Ten men receiving tetraiodothyroproprionic acid for 82 days failed to demonstrate any decrease in β-lipoprotein cholesterol. The hypocholesterolemic response to varying doses of desiccated thyroid indicated that many individuals without overt evidence of thyroid deficiency will develop sharp falls in β-lipoprotein cholesterol when they receive adequate replacement doses of desiccated thyroid. Data will also be presented upon the effect of the administration of other hormones with and without thyroid upon lipoprotein cholesterol. The significance of the interrelationships demonstrated by these studies in the development of atherosclerosis and aging will be discussed.

64. Modification of Experimental Atheromatosis by a Folic Acid Antagonist

Carl Muschenheim, Seymour Advocate, and Donald W. Hoskins. From the Department of Medicine, the New York Hospital—Cornell Medical Center, New York, N. Y.

It has been observed that atherosclerosis is often mild or absent in human cases of pernicious anemia. A program has therefore been undertaken to investigate a possible relationship between vitamin B₁₂ or folic acid metabolism to atherogenesis.

For the initial experiments aminopterin was selected as a readily available and easily administered folic acid antagonist. This drug, administered in the dose of 10 mg. per day subcutaneously to rabbits weighing 2-3 Kg., produces a profound and sometimes fatal anemia, but if a 2 day rest period is given each week the anemia may be maintained at an hematocrit level of 25-30 and the animals appear healthy and gain weight.

Rabbits pretreated with aminopterin for 4 weeks were fed an atherogenic diet for 8 weeks, during which aminopterin injections also were continued. The atherogenic diet consisted of a stock diet with 3/4 per cent cholesterol added. As a result of some manipulation of the aminopterin dosage during the period before cholesterol feeding was begun some of the aminopterin treated animals lost weight from overdosage and 7 of the original 20 died. The surviving animals upon reduction of dosage made good weight gains, comparable to the controls, during the cholesterol feeding period. When sacrificed after 8 weeks of cholesterol feeding the control animals, 21 in number, all had grossly demonstrable 1+ to 3+ atheromatosis of the aorta. Of the 13 surviving aminopterin treated animals 9 had no atheromatosis or doubtful deposits of minimal extent. Only 4 had definite atheromatosis comparable in extent to the controls. Serum cholesterol levels averaged 40 to 50 per cent lower in the treated animals than in the controls.

65. Comparative Incorporation of C¹⁴ from Labeled Acetate into Free Cholesterol, Fatty Acids and Estereified Cholesterol and Fatty Acids in the Calf Aorta


In humans, with advancing age, and in rabbits, with induced atherosclerosis, cholesterol esters showed a greater increase in concentration than did other lipids in a study to be published. Simultaneously, but as separate entities, incorporation of C¹⁴ from labeled acetate into the (a) free cholesterol, (b) some representative free fatty acids, and (c) the cholesterol and fatty acids comprising the cholesterol esters have been investigated to study this matter further.

Since the calf aorta actively synthesize both fatty acids and cholesterol it was chosen as the subject for 2 perfusion experiments employing whole blood as perfusate. Perfusion A was run for 8 hours. Perfusion B was also run for 8 hours with labeled acetate in the perfusate but then a second "unlabeled" perfusate was substituted for 24 hours.

The cholesterol esters of A and B were combined after silicie acid column. This was necessary since from both aortas a total of only 25 y
of cholesterol in the esterified form was obtained. The combined cholesterol esters were subjected to 3 chromatographic purifications. Since 20 mg. of free cholesterol were present in each aorta it was not requisite to combine these fractions.

The representative free fatty acid pool was obtained from the saponifiable fraction accompanying the free cholesterol. This fraction is free of triglycerides and phospholipids.

The results of the study indicate a five to eightfold greater degree of C14 incorporation from labeled acetate into free cholesterol and free fatty acids than in the cholesterol and fatty acids that comprise the cholesterol esters. The significance of this finding in the light of the earlier study will be discussed.

66. Butter, Corn Oil and Fibrinolysis in Rats

Robert M. O'Neal, Roosevelt L. Tillman and Wilbur A. Thomas. From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.

The production of coronary arterial thrombi and myocardial infarcts in rats on a high-fat diet including thiouracil (0.3 per cent), sodium cholate (2.0 per cent), butter (40 per cent), and cholesterol (5 per cent), has been reported previously. In the present experiment 3 groups of Wistar rats (19 rats in each group) were established in order to determine whether or not feeding the thrombogenic diet caused interference with streptokinase-activated fibrinolysis. The "butter" group was fed the above diet, the "corn oil" group was fed an identical diet except that corn oil was substituted for butter, and the "normal" group was fed a semi-synthetic diet similar to the others but without thiouracil, sodium cholate or cholesterol and with the only added fat being 2 per cent corn oil. Three rats, 1 from each group, were bled in sequence at 12 to 40 days and fibrinolysis tested on the citrated, recalcified plasma after the addition of lyophilized human plasma (Warner-Chilcott's Diagnostic Plasma) and streptokinase (Lederle's Varidase) solution in equal amounts to the three plasmas. The tests were performed with a "thrombelastograph."

The mean time necessary for complete clot-lysis was the same in the normal group and the corn oil group (28 minutes) but was significantly (p < 0.05) longer (76 minutes) in the butter group (the statistical method used was the analysis of variance of a randomized block).

These results suggest that the thrombogenic effect of the diet we have used in rats to produce coronary arterial thrombi and myocardial infarction is related to its inhibition of fibrinolysis.

67. Experiences with in vivo Production of Fibrinolysin Following Parenteral Administration of Nicotinic Acid in Humans

William B. Parsons, Jr. From the Department of Internal Medicine, Jackson Clinic and Foundation, Madison, Wis.

Intravenous injection of nicotinic acid (100 mg. in 5 minutes) in humans results in fibrinolytic activity in some instances, as demonstrated by observation of recalcified whole blood or plasma or by thrombelastographic tracings. Complete lysis of clots occurs in blood samples drawn as soon as 3 minutes or as long as 30 minutes after completion of the injection, but lesser degrees of lysis can sometimes be found in later specimens by observing tubes containing clots for 72 hours or by recording thrombelastograms for 16 to 24 hours.

The phenomenon is inhibited in patients who have been taking 3 Gm. or more of nicotinic acid daily for hypercholesterolemia. It is also inhibited for several hours after injection of nicotinic acid, failing to occur after a second injection. The frequency with which injections can be administered and consistently produce fibrinolysis has not yet been determined, and it is possible that daily or twice daily administration will result in failure to elicit this phenomenon in most patients.

Intramuscular injection of nicotinic acid (100 mg.) causes fibrinolysis in some patients but less consistently than intravenous administration. The effect of sublingual administration is currently being studied.

Nicotinamide injected parenterally fails to produce fibrinolytic effect, but its injection can inhibit the phenomenon when nicotinic acid is injected several hours later. Whether nicotinamide in multiple vitamin products taken days prior to testing can cause inhibition is uncertain. It is possible that patients receiving anticoagulants to reduce prothrombin concentration are less likely than others to develop fibrinolytic activity from nicotinic acid.

Addition of nicotinic acid to blood in vitro fails to result in fibrinolysis, regardless of whether it is added before or after coagulation occurs. Nicotinic acid blood levels, determined by bioassay, have thus far failed to show any consistent correlation with presence or absence of fibrinolysis in vivo following parenteral injection.

68. Validity of Serum Cholesterol Determinations on Postmortem Blood

J. C. Paterson and Lucy Dyer. From the Clinical Investigation Unit of Westminster Hospital, and the Collip Medical Research Laboratory, University of Western Ontario, London, Canada.
Landé and Sperry compared the postmortem level of serum cholesterol with the amount of lipid extracted from the aorta in a large series of individuals who died suddenly and violently. No significant relationship was thus obtained, but the validity of this result has been questioned because of the doubtful accuracy of cholesterol determinations on postmortem blood. We have examined this latter point during the past year.

Serum cholesterol determinations have been made serially during the last 5 years of life on 800 patients who are permanently confined to our hospital. Twenty-seven of these died during 1958-59, and on each we did an additional determination of the postmortem serum cholesterol level. The postmortem level was then compared with the mean antemortem level, with the pathologic findings, and with the clinical picture during the terminal illness.

In 20 cases the terminal illness was protracted and in these there were striking decreases (average 49 per cent) in the postmortem serum cholesterol levels compared with the antemortem levels. However, an entirely different result was obtained in persons who died suddenly and unexpectedly. In 6 out of 7 such cases, the postmortem levels were in the same range (2 per cent) as the mean antemortem levels. There was only 1 exception to this general rule: a 73-year-old man in apparent good physical health who died suddenly and unexpectedly. Postmortem examination revealed rupture of the heart through a recent myocardial infarct estimated to be about 10 days old. The average antemortem serum cholesterol in this case was 190 mg. per cent, and the postmortem level 134 mg. per cent. This case illustrates the complete unreliability of serum cholesterol levels determined during the acute stage of myocardial infarction.

In general, the results in this small series tend to support the claims of Landé and Sperry that postmortem serum cholesterol determinations are valid providing that death is sudden and unexpectedly and that no significant occult disease is revealed at autopsy. The corollary is that their 1936 report deserves more consideration than it has apparently received in the past.

69. Morphologic Changes in Atherosclerotic Plaques due to Exogenous Insulin Administration

Ruth Pick, and Louis N. Katz. From the Cardiovascular Department, Medical Research Institute, Michael Reese Hospital, Chicago, Ill.

It has been established that insulin inhibits diet-induced regression of coronary atherosclerosis in chicks. Recent experiments have demonstrated that insulin also counteracts the accelerated regression of lesions seen in estrogen-treated birds. The mechanism by which insulin operates is not known. However, histologic examination revealed significant alterations of the plaques in birds exposed to insulin. Eleven per cent of the insulin-treated animals and 47 per cent of insulin plus estrogen-treated animals showed an abundance of birefringent cholesterol crystals with only a very small amount of sudanophilic fat. This was not seen in control birds. Regular mash diet alone or regular mash plus estrogens in no instance produced this morphologic appearance. Ordinarily, the plaques induced during five weeks contain large amounts of sudanophilic fat with few, if any, cholesterol crystals. Evidently, insulin and/or insulin in combination with estrogen changes the character of the lipid deposit within the preexisting lesions. The significance of this phenomenon in relation to retardation of regression is not known.

70. Effects of Estradiol-17α in Males with Coronary Heart Disease

Roger W. Robinson, Norio Higano, and William D. Cohen. From the Research Laboratory and Medical Division, The Memorial Hospital, Worcester, Mass.

It was of interest to study the possible lipid-shifting effects of estradiol-17α, in view of its relative lack of estrogenicity in standard bioassays. Ten middleaged men with coronary heart disease were studied before and during oral administration of 10 mg. of estradiol-17α (AY-55102, Ayerst Laboratories) daily for 6 months. The following serum lipid parameters were checked monthly: total cholesterol, phospholipids, cholesterol/phospholipid ratio, α- and β-lipoprotein cholesterol by preparative ultracentrifugation, and the β/α-ratio. Clinical evaluation was obtained frequently to determine the development of the characteristic effects of estrogens in males. One patient developed marked decrease of libido after 2 months of administration despite an increase of the β/α-ratio from 5.6 to 7.5, an effect opposite to that typical of estrogens. Another patient complained of breast tenderness after 3 months, although the serum lipids remained at control levels. Of the remaining 8 men who completed the 6 months, 5 were free of estrogenic effects, while 3 developed late breast changes, one, breast hypertrophy and two, only breast tenderness. Monthly serum lipid determinations during this 6-month period showed no significant changes from control levels, whether or not estrogenic side-effects developed. Thus, estradiol-17α, while weakly estrogenic, proved to be devoid of any lipid-shifting properties at this dosage.
71. Human Pulse Rate during 24 Hours of Usual Activity

Donald A. Rowley, Seymour Glagov, and Peter Stoner. From the Department of Pathology, University of Chicago, Chicago, Ill.

Hemodynamic factors may account for the frequent localization of atheromatous plaques in the coronary arteries and the sparing of the renal arteries. Resistance to blood flow in the kidney is low; about 25 per cent of the cardiac output flows through the relatively small main renal arteries. Resistance to blood flow in the myocardium increases during systole; thus the main coronary arteries are unique in that they supply a tissue which increases resistance to blood flow during a portion of each cardiac cycle. According to Laplace's law, vessel wall tension is higher in the coronary artery than in the renal artery during each systole. The duration of systole remains constant at about 0.25 second within the range of normal pulse rates. With a low pulse rate increased coronary artery wall tension during systole may not be significant but with a sustained high pulse rate increased wall tension might account in part for the localization of atherosclerotic plaques in the coronary arteries.

A pulse rate for 24 hours, integrating the variations in minute rate produced by activity etc., might give useful information relating pulse rate to pathologic processes. An instrument for measuring 24 hr. pulse rates should be small, rugged, unencumbering, and self-contained. In cooperation with Illinois Bell Telephone engineers we have built an instrument measuring $9 \times 7 \times 1.5$ cm. and weighing 110 Gm. The electrical signal of each myocardial contraction, picked up from precordial electrodes and amplified, drives a cumulative counter. Electrodes which give an undistorted signal regardless of body position, physical activity, or trauma to the electrodes have been designed. The electrodes are easily applied and non irritating; they will not detach during strenuous exercise. The pulse counter is highly accurate during many kinds of activity. Preliminary data on 24 hour pulse rates will be discussed. The design of components of the counter will be presented.

72. Correlation of Activity Changes in Blood Coagulation Factors with Human Arteriosclerosis

Alfred C. Schram and L. O. Pilgeram. From the Arteriosclerosis Research Laboratory, St. Barnabas Hospital Research Foundation in cooperation with the Department of Physiology, University of Minnesota School of Medicine, Minneapolis, Minn.

A survey of the literature will reveal that information on whether blood coagulation factors change with the development of human arteriosclerosis is inadequate. The possible role of these factors in the etiology of arteriosclerosis and thrombosis points to the need for information on which factors change, if any.

A study was therefore undertaken of the changes which occur in coagulation factors with presence of human arteriosclerosis. All subjects fasted overnight for 8 or more hours. All factors were measured in the same sample of plasma from a given subject. Donors were divided into 2 groups: group A consisted of apparently healthy, young individuals (age range 22 to 28 years), while group B consisted of patients who had sustained a proven myocardial infarction at least 6 months prior to the blood drawing, and were without anticoagulant therapy for at least 5 months prior to the blood drawing (age range 36 to 74 years).

The citrated plasmas were analyzed for prothrombin; total antithrombin activity; antithrombins II and III; "stable component" (proconvertin); "labile component" (accelerator globulin); thromboplastin generation; antithromboplastic activity; fibrinogen; profibrinolysin; fibrinolytic and antifibrinolytic activities. The methods used were adapted from reported technics. Reagents were prepared only from normal human plasma and tissue, except in the case of stable component determinations, in which bovine proconvertin-free plasma was used. Test conditions were kept constant throughout the study.

The deviations of any single component within a group were large. Deviations ranged from 8 to 40 per cent from the mean for the group. However, the averages in both groups pointed to a definite increase in plasma clottability in the case of group B. Most significant were the increases in prothrombin level (34 per cent; $p = 0.03$); in thromboplastin generation (28 per cent; $p < 0.001$); and in fibrinogen level (34 per cent; $p = 0.01$). An appreciable increase in antithrombin II (19 per cent; $p = 0.06$) and in antithrombin III (33 per cent; $p < 0.002$) was also recorded. There was a trend for increased levels of stable component (14 per cent; $p = 0.25$); of labile component (38 per cent; $p = 0.25$); and of antithromboplastin (33 per cent; $p = 0.16$). In the same group, there was a slight trend for decreased values (about 10 per cent) in the levels of profibrinolysin, fibrinolysin and antifibrinolysin ($p = 0.12$).

73. Myocardial Necrosis Following Pitressin Administration in Dogs with Coronary Atheromatosis

Harry Sobel and Carl Monden. From the Institute for Medical Research, Cedars of Lebanon Hospital, and the Department of Biochemistry &
Nutrition, University of Southern California, Los Angeles, Calif.

The role of stress and emotionality as cofactors in the genesis of hypercholesterolemia, atheromatosis, atherosclerosis and myocardial and cerebral necrosis is under investigation in this laboratory. A reasonable hypothesis in regard to cardiac angina and necrosis would be that when coronary flow is already diminished because of atherosclerosis, further impairment resulting from mechanisms engendered by stress and emotionality would impede flow to such an extent as to cause hypoxic phenomena. A study was therefore undertaken of the effect of humoral agents which are known to be released following stress, upon dogs which had been maintained on an atherogenic regimen for periods up to 1 year. Two of 9 dogs receiving 0.3 units of commercial pitressin per Kg. body weight intravenously over a period of 20 seconds died within a few minutes. Those which survived received 2 additional injections and although electrocardiographic evidence of hypoxia was present in some, there were no further deaths. The 2 dogs which died exhibited severe coronary involvement while the 7 which survived had moderate or minimal coronary atheromatosis. One of the dogs which died had previously received numerous intravenous injections of norepinephrine (30 μg./Kg.) without permanent effect. There were no deaths in 10 normal dogs receiving these agents.

These preliminary findings suggest that the dog with experimental atheromatosis may be a useful tool for study of mechanisms which are activated by stress and emotionality and which may cause myocardial necrosis. Further studies with smaller doses of pitressin and other agents should be undertaken.

74. High Incidence of Myocardial Infarcts in Ulcer Patients Treated with Sippy and Other High-Milk Diets: A Study of Autopsied Patients in Fifteen Hospitals

Wilbur A. Thomas, Robert M. O’Neal, Richard D. Briggs, Martin L. Rubenberg, and W. Stanley Hartroft. From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.

In order to evaluate the role of milk products in the production of the high incidence of myocardial infarcts in patients with peptic ulcers, the incidence of myocardial infarcts has been determined for 3 groups of autopsied patients matched for age, sex, race, place, and period of death. The 3 groups of patients from 10 medical centers in the U.S.A. and 5 in Great Britain, are as follows:

1. Patients with peptic ulcers who had a history of treatment with the Sippy or other high-milk diet (Sippy-ulcer), (2) patients with peptic ulcers who were not known to have been so treated (non-Sippy-ulcer), and (3) nonulcer patients matched as indicated above with the other 2 groups. Statistically significant differences were found between the incidences of myocardial infaracts in the Sippy-ulcer patients and the incidences in either of the other 2 groups. These differences were apparent in patients from the hospitals in the U.S.A. as well as those from Great Britain.

In the U.S.A. the incidence of myocardial infarcts in 97 Sippy-ulcer patients was 36 per cent compared to 15 per cent in the other 2 groups (p = 0.01). In Great Britain, the incidences in 95 Sippy-ulcer patients was 18 per cent, 3 per cent greater than the incidence in the non-Sippy-ulcer (p = 0.01) and the 8 per cent incidence in the nonulcer patients (p = 0.05). No significant differences were present between incidences of myocardial infarcts in the non-Sippy-ulcer and nonulcer patients.

This relationship between the therapeutic dietary regimen and the incidence of infarction is beyond any statistical doubt, but these data only indicate a strong association between the 2; further studies would be necessary to establish any cause and effect basis.

75. Early Lesions in Rat Atherosclerosis

Bernard C. Wexler, George W. Kittinger, and Benjamin F. Miller. From the May Institute for Medical Research of the Jewish Hospital of Cincinnati and the Departments of Pathology and Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio.

Rats, bred repeatedly, developed arteriosclerosis spontaneously. The incidence and severity of these lesions may be increased by unilateral nephrectomy and injection of ACTH. Adrenal steroid analyses, adrenal histology, and thymic involution suggest participation of the pituitary-adrenal axis in the pathogenesis of the arterial damage. Lipids, demonstrable by the usual histological technics, did not appear except in the more advanced lesions; serum cholesterol levels remained essentially normal.

An attempt was made to locate and describe the earliest changes. Female and male rats (Sprague-Dawley), on a regular diet, were bred repeatedly and some sacrificed after each breeding. Other breeders were subjected to unilateral nephrectomy, treated with ACTH, or a combination of ACTH and nephrectomy. The earliest change observed was a subintimal accumulation of acid mucopoly-
saccharide (AMP) followed by fibrosis. Medial elastic fibers became swollen and were surrounded by AMP-positive granules. Next there appeared elastosis and pooling of mucopolysaccharides. In more advanced lesions, sites of dystrophic calcification were accompanied by lipid infiltration. Males and females differed in the morphologic development of atherosclerosis. Grossly detectable plaques appear in females by the fourth breeding, but not in the males.

ACTH and nephrectomy accelerated the arteriosclerosing process and produced unusual accumulation of mucopolysaccharides and heavy subintimal fibrosis. The aorta appears to be the primary "target organ," i.e., lesions appear first in the aorta and only secondarily in other organs.

76. Comparative Actions of Female and Male Sex Hormones on Aortic Atherosclerosis of Cockerels Fed a Cholesterol-Sugar Diet

Harry Y. C. Wong, Frank B. Johnson, Beatrice Liu and Rose Shim. From the Department of Physiology, Howard University Medical School, and the Armed Forces Institute of Pathology, Washington, D. C.

It has been reported that administration of estrogen to cholesterol fed chicks could reverse coronary atherosclerosis, but it had no significant effect on the aorta. Previous reports from our laboratory have shown that exercise and administration of male sex hormones to cockerels or capons significantly reduced blood cholesterol and aortic and coronary atherosclerosis in birds fed a diet of 2 per cent cholesterol and 5 per cent cottonseed oil. The present investigation was undertaken to study the effects of male and female sex hormones in reversing atherosclerosis of cockerels fed a diet of 2 per cent cholesterol and 5 per cent cottonseed oil. At the end of 10 weeks on this diet, 20 cockerels were sacrificed to determine the degree of aortic atherosclerosis. Hardly any atherosclerosis was seen, although there was a very significant increase in the blood cholesterol. Subsequently, cockerels were placed on a diet of 2 per cent cholesterol, 5 per cent cottonseed oil and 30 per cent sugar. Three groups of 10-week-old cockerels were used: Group 1, cockerels fed a 2 per cent cholesterol and 5 per cent cottonseed oil diet supplemented with 30 per cent sugar; group 2, similarly treated as group 1 except they were treated with 1.25 mg. testosterone propionate; and group 3, birds on a similar diet as group 1 except these were injected with 1.0 mg. of estradiol benzoate. After 7 weeks of treatment, it was observed that group 2 had the lowest blood cholesterol, and group 3 had a higher level than group 1 or 2. Upon autopsy, the testosterone-treated group had the least aortic atherosclerosis. This was statistically significant when compared to groups 1 and 3. Gross grading of the aortas of group 3 was higher than that of the controls.
CONTRIBUTORS TO THIS ISSUE

D. ALEKSANDROW, M.D.
Director, Second Clinic for Internal Diseases, Medical Academy, Warsaw, Poland.

ROBERT M. ANDERSON, M.D.
Instructor of Surgery, University of Southern California School of Medicine; Member of Staff, St. Vincent's Hospital, Los Angeles, Calif.

IVAN D. BARONOPBSKY, M.D.
Clinical Professor of Surgery, Columbia University College of Physicians and Surgeons; Surgeon-in-Chief, The Mount Sinai Hospital, New York, N.Y.

E. M. M. BESTERMAN, M.A., M.D., M.R.C.P.
Senior Registrar, the Middlesex Hospital, London, England.

HERRMAN L. BLUMGART, M.D.
Professor of Medicine, Harvard Medical School; Physician-in-Chief, Beth Israel Hospital, Boston, Mass.

EUGENE BRAUNWALD, M.D.
Chief, Section of Cardiology, Clinic of Surgery, National Heart Institute; Lecturer in Physiology, George Washington University, Washington, D.C.

BERNARD L. BROFMAN, M.D.
Consultant, Hexter Cardio-Pulmonary Laboratory; Senior Visiting Physician, Mount Sinai Hospital of Cleveland, Cleveland, Ohio.

TIMOTHY N. CARIS, Lt. Col., USAF (MC)
Chief, Cardiology Service, USAF Hospital Scott, Scott Air Force Base, Ill.

BERNARD L. CHARMS, M.D.
Director, Hexter Cardio-Pulmonary Laboratory, Mount Sinai Hospital of Cleveland, Cleveland, Ohio.

HARVEY COHEN, M.B., B.CH.
Assistant Pediatrician, Coronation Hospital and University of the Witwatersrand, Johannesburg, South Africa.

T. J. DANARAJ, M.D., M.R.C.P.E.
Senior Lecturer in Medicine, University of Malaya, Singapore; Consultant Physician, General Hospital, Singapore.

ROBERT B. DICKERSON, COL., MC, USA
Presently, Chief, Cardiology Service, USAF Hospital Scott, Scott Air Force Base, Ill.

JAMES W. DUSHALE, M.D.
Consultant, Section of Pediatrics, Mayo Clinic; Associate Professor of Pediatrics, Mayo Foundation, Graduate School, University of Minnesota, Rochester, Minn.

DAVID G. FREIMAN, M.D.
Clinical Professor of Pathology, Harvard Medical School; Pathologist-in-Chief, Director of Laboratories, Beth Israel Hospital, Boston, Mass.

CHARLES R. GREENE, M.D.
Chief Resident in Medicine, Kings County Medical Center, Assistant Instructor in Medicine, Department of Medicine, State University of New York, Downstate Medical Center, Brooklyn, N. Y.

GEORGE C. GRIFFITH, M.D.
Professor of Medicine (Cardiology), University of Southern California School of Medicine, Los Angeles, Calif.

BETTY HAHNEMAN, M.D.
Instructor in Medicine, Northwestern University, Chicago, Ill.

ANTONI HORST, M.E.
Director, Department of Physiopathology, Medical Academy, Poznań, Poland.

JOHN H. JUHL, M.D.
Consultant, Radiological Service, Veterans Administration Hospital; Associate Professor Radiology, University Hospital, Madison, Wis.

994 Circulation, Volume XX, November 1959
CONTRIBUTORS TO THIS ISSUE

JEROME HAROLD KAY, M.D.
Associate Professor of Surgery and Chief of Cardiac Surgery, University of Southern California School of Medicine; Chief of Heart Surgery, St. Vincent's Hospital; Head Physician, Thoracic Surgery, Los Angeles County General Hospital, Los Angeles, Calif.

JOHN J. KELLY, JR., M.D.
Associate Professor of Medicine, State University of New York, Downstate Medical Center, Brooklyn, N. Y.

JOSEPH R. KELLY, M.D.
Resident, Internal Medicine, Veterans Administration and University Hospitals, Madison, Wis.

PAUL M. KOHN, M.D.
Associate, Hexter Cardio-Pulmonary Laboratory; Assistant Visiting Physician, Mount Sinai Hospital of Cleveland, Cleveland, Ohio.

ISADORE KREEL, M.D.
Trainee, National Heart Institute, N.I.H., U.S.P. H.S. (Grant #81-494), The Department of Surgery, The Mount Sinai Hospital, New York, N. Y.

SUSAN C. LENKEI, M.D.
Travelling Fellow of the R. S. McLaughlin Fellowship Foundation, University of Toronto, Toronto, Ontario, Canada, assigned to the Mayo Foundation, Graduate School, University of Minnesota, Rochester, Minn.

MORLEY LERTZMAN, M.D.
Formerly, Research Fellow in Medicine, Harvard Medical School; Research Fellow in Medical Research, Beth Israel Hospital, Boston, Mass.; Research Fellow, Massachusetts Heart Association.

REUBEN LEWIS, M.D.
Member of Staff, St. Vincent's Hospital, Los Angeles, Calif.

ROBERT T. L. LONG, M.D.
Senior Assistant Surgeon, Clinic of Surgery, National Heart Institute, Bethesda, Md.

OSCAR MAGIDSON, M.D.
Director, Cardio-Respiratory Laboratory, St. Vincent's Hospital; Associate Clinical Professor of Medicine, University of Southern California, Los Angeles, Calif.

CARL MARIENFELD, M.D.
Chief, Program Services Section, Heart Disease Control Program, Division of Special Health Services, U. S. Public Health Service.

JOHN MEIHAUS, M.D.
Member of Staff, St. Vincent's Hospital, Los Angeles, Calif.

ROBERT A. MILLER, M.D.
Assistant Professor of Pediatrics, Northwestern University Medical School; Cardiologist, Children's Memorial Hospital, Chicago, Ill.

ANDREW G. MORROW, M.D.
Chief, Clinic of Surgery, National Heart Institute, Bethesda, Md.

Senior Registrar, Department of Cardiology, the Middlesex Hospital, London, England.

WONG HEE ONG, M.B., B.S.
Medical Officer, General Hospital, Singapore.

WILLIAM W. PFAFF, M.D.
Senior Assistant Surgeon, Clinic of Surgery, National Heart Institute, Bethesda, Md.

BERTRAM PITT, M.D.
University of Basel, Switzerland; Presently, Intern in Medicine, Beth Israel Hospital, New York, N. Y.

HOWARD K. RASMUSSEN, B.S.
Chief Technician, Pulmonary Function Laboratory, Veterans Administration Hospital, Madison, Wis.

STANLEY M. REIMER, PH.D.
Research Fellow in Medicine, Harvard Medical School; Research Fellow in Medical Research, Beth Israel Hospital, Boston, Mass.

LEOPOLD REINER, M.D.
Visiting Associate Professor of Pathology, Albert Einstein College of Medicine; Pathologist and Director of Laboratories, The Bronx Hospital; New York, N. Y.

ROBERT F. RUSHMER, M.D.
Professor of Physiology, Department of Physiology and Biophysics, University of Washington School of Medicine, Seattle, Wash.
CONTRIBUTORS TO THIS ISSUE

SHIRLEY SIEW, M.B., B.CH.
Senior Lecturer, Department of Pathology and Microbiology, University of the Witwatersrand, Johannesburg, South Africa.

JACQUES M. SMITH, M.D.
Associate in Medicine, Northwestern University, Chicago, Ill.

HAROLD J. SOBEL, M.D.
Eugene Meyer, Jr., Fellow in Pathology, The Department of Pathology, The Mount Sinai Hospital, New York, N. Y.

WALTER SOMERVILLE, M.D., F.R.C.P.
Lecturer in Cardiology, the Middlesex Hospital Medical School; Cardiologist, Thoracic Surgical Unit, Harefield Hospital, Middlesex, England; Assistant Physician, Department of Cardiology, the Middlesex Hospital, London, England.

HAMILTON SOUTHWORTH, M.D.
Clinical Professor of Medicine, College of Physicians and Surgeons, New York, N. Y.

ISAAC STARR, M.D.
Hartzell Research Professor of Therapeutics, School of Medicine, University of Pennsylvania, Philadelphia, Pa.

H. J. C. SWAN, M.B., PH.D., M.R.C.P. (LOND.)
Consultant, Section of Physiology, Mayo Clinic; Assistant Professor of Physiology, Mayo Foundation, Graduate School, University of Minnesota; Rochester, Minn.

R. H. WASSERBURGER, M.D.
Chief of Cardiology, Assistant Chief of Medicine, V.A. Hospital; Assistant Professor Clinical Medicine, University Hospital, Madison, Wis.

STANFORD WESSLER, M.D.
Assistant Professor of Medicine, Harvard Medical School; Visiting Physician and Physician to the Vascular Clinic, Beth Israel Hospital, Boston, Mass.; Established Investigator, American Heart Association.

JOEL WILLARD, B.S.
Joel Willard Productions, Chicago, Ill.

LAWRENCE I. ZAROFF, M.D.
Research Fellow, Dazian Foundation, Department of Surgery, the Mount Sinai Hospital, New York, N. Y.