Evaluation of Serum Lipoprotein and Cholesterol Measurements as Predictors of Clinical Complications of Atherosclerosis

Report of a Cooperative Study of Lipoproteins and Atherosclerosis

Circulation

OCTOBER, 1956
VOL. XIV NO. 4
Part Two

OFFICIAL JOURNAL of the AMERICAN HEART ASSOCIATION

Evaluation of Serum Lipoprotein and Cholesterol Measurements as Predictors of Clinical Complications of Atherosclerosis

Report of a Cooperative Study of Lipoproteins and Atherosclerosis

By the joint efforts of The Technical Group of the Committee on Lipoproteins and Atherosclerosis: JOHN W. GOFMAN, MARTIN HANIG, HARDIN B. JONES, MAX A. LAUFFER, ELEANOR Y. LAWRY, LENA A. LEWIS, GEORGE V. MANN, FELIX E. MOORE, FREDERICK OLMSTED, AND J. FRANKLIN YEAGER; *and The Committee on Lipoproteins and Atherosclerosis of the National Advisory Heart Council:* E. COWLES ANDRUS, J. H. BARACH,* J. W. BEAMS, JOHN W. FERTIG, JOHN W. GOFMAN, MAX A. LAUFFER, IRVINE H. PAGE, JAMES A. SHANNON, FREDRICK J. STARE, AND PAUL D. WHITE

IN 1950 the attention of the National Advisory Heart Council was called to studies by Gofman and his colleagues at the Donner Laboratory of the University of California.[1] These workers applied a new biophysical technic to a study of the sequence of lipid changes in the sera of rabbits fed cholesterol and also in the sera of human beings believed to have coronary atherosclerosis. They stated, "Some two years ago the present group of authors undertook a physiochemical investigation of those giant molecules of serum which may be composed of cholesterol, its esters, phospholipids, fatty acids, and protein as building blocks. The basic premise was that it is entirely possible that a defect might exist in certain of these giant molecules, which could be responsible for the development of atherosclerosis, whereas the mere analytic levels of any of the building blocks in serum might be of little or no significance." This approach to the problem of atherosclerosis seemed promising.

A summary of this work was brought to the attention of the National Advisory Heart Council by Dr. Shields Warren, then director of the Division of Biology and Medicine of the Atomic Energy Commission, which had sponsored the research in the Donner Laboratory. The Heart Council was impressed with the potentialities of these experimental findings and so appointed an *ad hoc* committee† for review of this work in the Donner Laboratory. The Heart Council then appointed a planning committee to formulate a program of action.‡ The

This investigation was supported by research grants from the National Heart Institute, U. S. Public Health Service.

* Deceased.

† Drs. Irvine H. Page, Hans Neurath, Forrest Kendall, and James A. Shannon.

‡ Drs. Paul D. White, E. Cowles Andrus, J. H. Barach (deceased, March 1954), John W. Gofman, Irvine H. Page, and James A. Shannon. Drs. Max A. Lauffer, Fredrick J. Stare, John W. Fertig, and J. W. Beams joined the committee at later times. Dr. C. J. Van Slyke, who was then director of the National Heart Institute, was active in the initiation of the Cooperative Study and the organization of this committee.

planning committee was designated as the permanent "Committee on Lipoproteins and Atherosclerosis" for the purpose of advising the Heart Council. It was decided that the research task could be best undertaken with the coordinated efforts of several research centers. On the recommendation of this planning committee, the Heart Council recommended research grant support for a field study to be carried out by several laboratories cooperatively.

Organization

The cooperative research was done by 4 laboratories with the research grant funds recommended by the Heart Council and with statistical and administrative assistance made available by the National Heart Institute. The recipients of these grants were Dr. John W. Gofman of the Donner Laboratory, University of California (Donner)*; Dr. Irvine H. Page, Research Division, Cleveland Clinic Foundation (Cleveland); Dr. Max A. Lauffer, Department of Biophysics, University of Pittsburgh (Pittsburgh); and Drs. Fredrick J. Stare and George V. Mann, Department of Nutrition, Harvard School of Public Health (Harvard). Mr. Felix E. Moore of the Biometrics Research Section, National Heart Institute served as statistical consultant to the committee and Dr. J. Franklin Yeager, Grants and Training Branch, National Heart Institute served as administrative officer. The Committee designated a Technical Group† composed of those immediately concerned with the work of the study.

Objectives

The cooperative effort was directed toward 5 objectives‡:

1. "To determine the range of values of the S_f 12–20 fraction (lipoprotein) in 'normal' persons of all ages in both sexes."
2. "To obtain further confirmation of the existence of a correlation between an elevated S_f 12–20 fraction in the blood of 'normal' persons and the subsequent development of myocardial infarction."
3. "To determine more fully the effects of a low-fat, low-cholesterol diet upon the S_f 12–20 fraction in patients with myocardial infarctions and upon further history of the disorder."
4. "To compare total cholesterol and S_f 12–20 fraction as indicators of the disorder."
5. "To relate the S_f 12–20 fraction and total cholesterol not only in normals and myocardial infarction patients but also in hypertensives and diabetics, especially in patients that develop myocardial infarctions."

The studies of the first of these objectives are reserved for a separate report dealing with the biology of serum lipoproteins and cholesterol. The present report deals only with the second and fourth objectives. Because of difficulties in establishing objective criteria for the selection of cases it was agreed to abandon the attempt to study cooperatively the effects of dietary treatment (objective 3) and the distribution of serum lipid levels in patients with overt disease (objective 5). It was agreed that the cooperating laboratories should publish their findings in these fields individually, or perhaps reach separate agreements for merging their findings.

The present report is concerned with the relation of lipoprotein and cholesterol levels to the appearance of cardiovascular events in previously well subjects. These studies have led to a critical evaluation of the predictive value of each of these measurements in relation to the development of clinical events attributable to atherosclerosis.

Plan of the Study

For these purposes the Lipoprotein Committee assumed that it would be necessary to study approximately 100 "new events." From an estimated annual incidence of myocardial infarction in apparently well American men aged 40–59 of 10/1000, it followed that a base population of 10,000 persons would yield this number of new events in 1 year of clinical follow-up. In order to secure this large number of subjects (and there were an additional 4000

* The laboratory designations in parenthesis will be used hereafter.

† John W. Gofman (chairman), Martin Hanig, Hardin B. Jones, Max A. Lauffer, Eleanor Y. Lawry, Lena A. Lewis, George V. Mann, and Frederick Olmsted. J. Franklin Yeager served as the administrative officer of the group and Felix E. Moore as the statistical adviser.

‡ Quoted from the History and Summary of the Committee on Lipoproteins and Atherosclerosis, February 26, 1952. This manuscript was prepared by J. Franklin Yeager and is a condensation of the progress and actions of the study.

subjects needed for study of other objectives) it was clear that the clinical criteria for admission to the study could not be rigorous. The principal sources of subjects were expected to be industrial groups in which individuals would qualify either with a preemployment type of examination or an annual health protection type of examination given by the employer. The cooperating laboratories did not attempt to carry out these clinical evaluations.

With this organization and these plans, the 4 laboratories have measured the serum lipids of approximately 15,000 subjects in a period of about 3 years. There were 4,914 men in the age group 40–59 who were judged to be clinically normal (according to the criteria of the study) at time of entry for whom a clinical follow-up was completed at the end of either 1 or 2 years. Within this group there were 82 individuals who developed evidence of new disease which, in the opinion of an independent Review Committee, was attributable to atherosclerosis. The present report is concerned with the relation of the lipid measures in this small group who developed new disease to the lipid measures in the presumably normal group out of which they arose.

Methods

Population

The geographic distribution of the cooperating laboratories fostered a desirable scattering of the sources of subjects across the United States. Since participation was arranged locally, the laboratories tended to study subjects obtained in nearby sources although some arrangements led to overlapping of territories. A tabular description of the sources according to the numbers of subjects, type of industry and of employment, and method of selection is shown in table 1. The sources were largely industrial organizations, but they ranged from penal to academic groups. The number of clinically normal men finally accepted from the separate sources varied from 4 to 837. The individuals studied represented many types of employment and included executive, clerical, sales, and labor personnel who appeared in the clinics for an annual, company sponsored examination.* While these examinations were not always mandatory, they were generally well accepted by the personnel. The Cleveland Graphite Bronze and Swift and Co. personnel were volunteers for this particular examination and there were variable participation rates. The Framingham Heart Study[2] and the Los Angeles Civil Service Study[3] provided a large proportion of the subjects of the Donner Laboratory. Each of these is a long-term epidemiologic study based on a random sample in which extensive clinical data have been carefully gathered for other research purposes.

Serum Methods

The methods devised by Gofman and his colleagues for the measurement of serum lipoproteins have been described.[4] Although the original design and historical application of the ultracentrifuge were for qualitative distinction of materials, with appropriate technical methods it has been successfully applied to the problem of measurement of concentrations of lipoproteins and proteins. The work with lipoproteins was complicated by lack of a suitable reference or calibration material capable of preservation in a constant state. Recognizing many of the technical difficulties, Gofman invited the Committee to send a member of the professional staff of each participating laboratory to the Donner Laboratory for a short period of apprenticeship to familiarize him with the technics. This training was carried out in the fall of 1950. These original laboratory procedures were in use throughout the study with certain minor modifications that are described in later sections and that were uniformly introduced in each laboratory.

The clinic personnel were instructed to collect venous blood in a clean, dry syringe without regard

* The employees of the White Motor Co. studied by Cleveland were not subjected to physical examination either on admission to the study or at follow-up, but were admitted as volunteers who were known to have good employment attendance records for periods of 1 to 15 years and in addition denied symptoms of serious illness. Because of this variation in clinical assessment, the results for this group have been presented separately.

TABLE 1.—*Groups Studied: Summary Description*

Laboratory and group	Location	Number of subjects*	Type of industry	Type of employment	Method of selection
Cleveland					
White Motor Co.	Cleveland, Ohio	235	automotive	salary and wages	volunteers
Chrysler Motor Co.	Detroit, Mich.	464	automotive	salary and wages	vol. clinic visits
Cleve. Grph. Bronze	Cleveland, Ohio	264	foundry	salary and wages	volunteers
General Electric Co.	Schenectady, N. Y.	183	electrical	salary	annual exam
Nickel Plate Railroad	Cleveland, Ohio	63	railroad	salary	annual exam
Donner					
Framingham Heart Study	Framingham, Mass.	837	townspeople	varied	sample survey
City of Los Angeles	Los Angeles, Calif.	686	civil service	office and labor	sample survey
Pan American Airways	San Francisco, Calif.	130	airline	salary and wages	annual exam
United Airlines	San Francisco, Calif.	64	airline	salary and wages	annual exam
Eastman Kodak Co.	Rochester, N. Y.	371	chemical	salary and wages	annual exam
Harvard					
Am. Mutual Ins. Co.	Boston, Mass.	9	insurance	salary and wages	annual exam
Dr. Burwell	Boston, Mass.	6	electronics, textiles	salary	annual exam
Campbell Soup Co.	Camden, N. J.	5	food	salary	volunteers
Dr. Chapman	Boston, Mass.	14	varied	salary	annual exam
Lahey Clinic	Boston, Mass.	136	banking, small industry	salary	annual exam
Metropolitan Life Ins. Co.	New York, N. Y.	534	life insurance	clerical, maintenance	annual exam
Mass. Inst. of Technology	Cambridge, Mass.	76	academic	faculty	annual exam
Rexall Drug Co.	L. A., St. Louis & Atlanta	10	pharmaceutical	salary	annual exam
Standard Oil of N. J.	New York, N. Y.	7	petroleum	salary	volunteers
Swift and Co.	Chicago, Ill.	39	meat packing	salary	volunteers
Oxford Diabetes Study	Oxford, Mass.	7	townspeople	varied	volunteers
Pittsburgh					
Hoffman-LaRoche	Nutley, N. J.	201	pharmaceutical	salary and wages	annual exam
Weirton Steel Co.	Weirton, West Va.	367	steel	salary and wages	annual exam
Federal Prisons:	Atlanta, Ga.	184	penal	staff and inmates	volunteers
	Leavenworth, Kan.	158	penal	staff and inmates	volunteers
	Terre Haute, Ind.	52	penal	staff and inmates	volunteers
Westinghouse Electric Co.	Pittsburgh, Pa.	4	electrical	salary and wages	annual exam
Armco Steel Corp.	Butler, Pa.	17	steel	salary and wages	annual exam
Ford Engineering Co.	Dearborn, Mich.	26	automotive	salary and wages	annual exam

* Includes only men, 40–59, with completed follow-up who were normal at entry to the study.

for the time of the last food ingestion.* The clotted blood was then centrifuged and the serum removed and stored at 0–5 C. No preservatives were used and the material was not frozen before the lipoprotein measurement. Grossly lipemic sera were mixed by inversion to obtain at least a crude suspension before removing aliquots. The sera were sometimes frozen and stored for variable times before the cholesterol measurement was done, but this practice was not uniform among the laboratories. No lipoprotein measurements were done on frozen samples.

Serious consideration was given to a plan for storing large numbers of sera drawn from healthy subjects and then, after a subsequent clinical evaluation had revealed the subjects with new disease, carrying out lipid analyses with only the sera of the

* Blood samples were drawn from the subjects in the Los Angeles civil service population when they arrived at the clinic in the morning after having been instructed to omit breakfast. This group approximated the postabsorptive state.

people with new disease and a sample of the remaining base population. This procedure, which would have reduced the laboratory work of the study by about 95 per cent, was impracticable because of the instability of serum lipoproteins with freezing or after long storage in solution. A joint study of the effects of storage of whole serum was carried out and indicated that this could be stored at 0–5 C. for periods up to 28 days without significant alteration of the lipoprotein pattern or content. In practice, the analyses were almost always begun within a week of the bleeding date.

The Spinco Company established in 1951 that the substitution of a 0.01 inch wire for the 1/32-inch bar initially used as the resolving element in the Schlieren system of the ultracentrifuge would improve the legibility of pattern photographs and would also resolve the technical difficulty of accounting for the attenuation of the wide bar shadow. The latter required an allowance in estimating the image position during interpretation of film patterns. Accordingly all laboratories converted to the wire elements on November 20, 1951, and either appropriate corrections or re-evaluations of films were made for values previously obtained with the bar elements in order to make these earlier measurements equivalent.

These and other technical matters were discussed in detail in the proceedings of a symposium entitled "Lipoproteins and Ultracentrifugal Technique,"* which was sponsored by the Technical Group and held in Chicago on November 8, 1952.

Lipoprotein Nomenclature

The classical notation that was applied to the unit of sedimentation (S)† in honor of Svedberg[5] was appropriate to sedimenting materials but a description of rates of motion in the opposite direction, i.e., with flotation, required either the use of negative S or the invention of some new notation. Gofman and his colleagues designated flotation rates with S_f.[1] In the present work the conditions are medium density 1.063, temperature 26±1 C. and with a medium viscosity of a 9.37 per cent (Gm./ml. solution) solution of sodium chloride in water. The calculation of concentration is based upon an assumed specific refractive increment of 1.54×10^{-3} for β-1 lipoprotein‡ with the presumption that the low-density lipoproteins under study would have refractive increments of similar value.

In the original work, the Donner group made certain arbitrary subdivisions of the low density lipoproteins of sera according to the flotation rates in S_f units. These divisions were intended to separate several bands of lipoproteins so that they might be tested for relationship with certain diseases and particularly with atherosclerosis. In the experiments with rabbits, for example, they found that ". . . those rabbits failing to develop high levels of the components of S_f greater than 5–8 units showed no gross atherosclerosis or showed minimal atherosclerosis, whereas mild to severe atherosclerosis developed in those with high concentrations of the molecules of the S_f 10–30 class."[1] In the same report, the following conclusions were drawn from studies with human subjects: "Study of several groups of individuals with respect to the presence of molecules of the S_f 10–20 class indicates that the presence of these molecules in the serum of humans is related to the development of atherosclerosis." The initial concern of the Cooperative Study was with the S_f 10–20 band of low-density lipoproteins and the relation of these materials in the serum to atherogenesis. In July 1951 the Donner group proposed[8] that the proper measurement was of the S_f 12–20 band, since this separation would better differentiate the major β-lipoprotein material in sera (S_f 0–11) from the lower density material believed more pertinent to the development of atherosclerosis. A little later[9] the Donner group proposed another modification of the hypothesis to include the S_f 20–100 band in the belief that this material was also of importance to atherogenesis. The Technical Group with the agreement of the Committee admitted these modifications, since the added

* The mimeographed proceedings may be obtained from J. Franklin Yeager, National Heart Institute, National Institutes of Health, Bethesda, Maryland.

† S = Svedberg = 1×10^{-13} cm./sec./dyne/Gm. This is a unit of a rate of motion under specific conditions.

‡ This constant, the change of the index of refraction of a solution caused by a per cent change of concentration of the lipoprotein, measures the extent to which these particles contribute to the refraction of light by a solution. The observed deflection of the image of the resolving element depends upon this quantity. Recent measurements of this constant by Hanig and Shainoff[6] for β-1 lipoprotein separated by flotation indicate that the value is 1.51 $\times 10^{-3}$ and that the estimate of Armstrong and coworkers[7] that is used here is substantially correct.

TABLE 2.—*First Preliminary Reproducibility Study, April 1951. 18 Specimens Measured in Duplicate by Each Laboratory (Levels in mg./100 ml. Serum)*

Laboratory	S_f 10–20		Total cholesterol		
	Mean	σ_e	Mean	σ_e	Method*
Cleveland	36.5	9.00	263	24.60	Abell
Donner	50.8	7.76	229	13.75	Coleman
Harvard	36.2	6.80	200	20.34	Hemolytic
Pittsburgh	49.5	13.90	250	7.74	Hemolytic
Framingham	—	—	219	10.69	S-S

* *Abell:* A then newly developed method that was kindly supplied by the authors in preprint form. The method has subsequently been described in the literature.[14]

Coleman: A method developed at Donner Laboratory.[15]

Hemolytic: This method was devised and used in the Harvard laboratory by White and Mann. It was not published in the literature and has since been abandoned.

S-S: The method of Schoenheimer and Sperry.[16a, b]

measurements were readily available without major alterations of the established procedures. The Cooperative Study is thus concerned with the validity of the hypothesis that relates the S_f 12–20 and S_f 20–100 bands of low-density lipoproteins to coronary atherogenesis and the relative merits of measurement of this material and of the total cholesterol in reflecting this relationship.

The Donner group in 1952 showed that lipoprotein measurements under the conditions being used in the Cooperative Study should be corrected for the effects of concentration on flotation rates.* When such corrections are made, the designation of *Standard flotation* rates (units = S_f^0) is applied to the measurements. The original studies upon which the hypothesis of a relationship of lipoproteins with human atherosclerosis was based were conducted under conditions such that the lipoproteins were concentrated threefold over their original concentration in serum.[1] When the cooperative study was initiated, a fivefold concentration of lipoproteins was being used.[11] This modification had been introduced in the desire to reduce measurement errors for low concentrations of lipoproteins by having greater concentrations in the analytic ultracentrifuge cell. At that time the magnitude of flotation rate dependence upon concentration was not appreciated. Later studies by the Donner group indicated that serious errors in the measured S_f 12–20 lipoprotein concentrations could arise in samples of high total concentration of lipoproteins, unless the correction for flotation concentration dependence was made. The Donner group proposed that all cooperating laboratories undertake the more refined measurement to eliminate this source of error. The other laboratories were unable to undertake this revision.

The Donner group has extended the measurement base of the original hypothesis to include the "standard" measurements, S_f^0 0–12 and the S_f^0 100–400,[12] and have combined the "standard" measurements into indices, some of which have included additional variables such as age[12] and diastolic blood pressure.[13] The derivation and the interpretation of these multivariable indices are not an immediate concern of the Cooperative Study, and they will be referred to only in the section headed Discussion. It should be appreciated that *only* the Donner Laboratory has carried out these refined (S_f^0) and extended (S_f^0 0–12 and S_f^0 100–400) measurements so that the other laboratories in the Cooperative Study cannot bring independent evidence to bear on these new hypotheses.

Appraisal of Methods

In the beginning the 4 laboratories used 3 different cholesterol methods. It seemed unlikely that equivalent data would be obtained without a systematic study of the performance of the several laboratories. The technical complexity of both the cholesterol and lipoprotein methods also required some kind of continuing calibration among the laboratories. An equivalence of technical performance was obviously necessary if the data were to be pooled for evaluation as was contemplated.

A reproducibility study of both cholesterol and lipoprotein measurements was carried out in April 1951 for these reasons. Serum samples were supplied each laboratory as coded duplicates and were analyzed by operators who were unaware either of the presence of check samples or of the identity of pairs. The results of this study are shown in table 2.

* This was first reported to the Technical Group at its meeting on June 11, 1952.[10]

The laboratory personnel of the Framingham Study* participated in the cholesterol analysis because they were using the Schoenheimer-Sperry method,[16a,b] which has some repute as a "standard method." The results of this study revealed intolerable differences between laboratories in measuring cholesterol both according to the mean levels and the technical error in measurement.† The measurement of S_f 10–20 (done before the S_f 12–20 and 20–100 modifications described above were introduced) also showed lack of equivalence among laboratories. The results of a second study of cholesterol measurement are shown in table 3. Drs. Liese Abell and Forrest Kendall of the Goldwater Memorial Hospital, New York City, participated in this and some of the later trials.

An extensive series of studies was then begun in an effort to minimize or remove these laboratory differences. Samples of whole and partially processed sera, ultracentrifuge cells, and film patterns were exchanged among the laboratories, details of methods were examined and within-laboratory testing of reproducibility was established. The latter procedure consisted of the introduction of blind, duplicate samples into the routine work of each laboratory. The values for these duplicate runs were reported at intervals to the statistical consultant. They are shown in summary in table 4. Among other findings, these studies revealed an error in the lipoprotein measurements at Donner that was traced to a misalignment of analytic ultracentrifuge cells in the rotor. This had resulted from an error in the construction of certain cells made and used in that laboratory. These cells were replaced with Spinco cells and the measurements obtained with the defective cells were not included in the study.

Persistent difficulties with cholesterol methods during 1951 led to the adoption of the Abell method.[14] This procedure was used after January 1952 in all except the Donner Laboratory. In that laboratory, repeated evaluations of the Abell and Coleman methods[15] were done and these indicated that measurements by the Coleman method corresponded more closely with those done in other laboratories than did measurements by the Abell method. Accordingly, the Donner Laboratory continued with the Coleman method. The Pittsburgh Laboratory using the Abell Method did not obtain the same mean values of serum cholesterol that were obtained by the other laboratories. The means of Pittsburgh were about 10 per cent lower than the others throughout the range of levels encountered.

General reproducibility tests were begun on a regular schedule in January 1952. These tests were carried out during 15 of the next 18 months and the final test was done in June 1953. The laboratories alternately supplied 10 coded samples of sera or citrated

TABLE 3.—*Second Preliminary Reproducibility Study, October 1951, Fourteen Specimens Measured in Triplicate by Each Laboratory (Levels in mg./100 ml. serum)*

Laboratory	Total cholesterol		
	Mean	σ_e	Method*
Cleveland	215	4.04	Abell
Donner	203	5.54	Coleman
Harvard	215	20.67	Hemolytic
Pittsburgh	255	23.09	Hemolytic
Goldwater	216	3.26	Abell

* See notes, table 2.

TABLE 4.—*Summary of Within-Laboratory Reproducibility Studies, September 1951–June 1953, Levels in mg./100 ml. Serum*

	Cleveland	Donner	Harvard	Pittsburgh
S_f 12–20				
Period	9/51–4/53	9/51–6/52	9/51–6/53	9/51–10/52
Number of paired measurements	374	171	618	174
Technical error	4.7	4.7	5.1	5.5
S_f 20–100				
Period	10/51–4/53	10/51–6/52	10/51–6/53	10/51–10/52
Number of paried measurements	295	144	567	113
Technical error	11.0	12.3	9.6	9.9
Total cholesterol				
Period	9/51–4/53	9/51–6/52	1/52–6/53	1/52
Number of paired measurements	514	194	512	19
Technical error	7.6	17.3	10.5	5.0

* T. R. Dawber, M.D., Director, and Janet Hill, Chief Technician.

† The technical error of measurement is $\sigma_e = \sqrt{\Sigma d^2/2k}$ i.e., the square root of the sum of the squared differences of duplicates divided by twice the number of pairs. This statistic will be used as a measure of duplicate reproducibility. If the assumption is made that distribution of duplicate differences is normal and the errors of all pairs can be pooled, a single observation would be expected to differ from its true value by more than plus or minus σ_e in less than one third of the trials.

TABLE 5.—*Summary of Between-Laboratory Reproducibility Studies, January 1952–June 1953, Total Cholesterol, Levels in mg./100 ml. Serum*

Date	No. of specimens measured in duplicate	Mean lipid values						Technical errors σ_e				
		Cleveland	Donner*	Harvard	Pittsburgh	Goldwater†	Framingham‡	Cleveland	Donner*	Harvard	Pittsburgh	Goldwater†
1952												
January	10	230	221	226	213	—	—	4.2	4.1	3.9	2.7	—
February	10	215	208	201	207	—	—	3.8	5.8	2.7	3.3	—
March	10	205	205	206	197	—	—	5.0	—	9.7	6.3	—
April	10	178	173	183	164	—	—	3.4	3.5	5.5	3.3	—
June	10	166	155	157	153	158	—	3.1	5.9	3.6	3.8	3.4
July	10	165	165	169	154	163	—	3.9	3.8	6.6	4.6	2.5
September	10	229	236	221	204	227	—	2.1	5.2	4.3	4.0	2.5
October	10	233	225	224	200	229	225	2.3	6.3	3.5	5.6	5.4
December	10	176	195	192	169	188	180	4.3	4.8	4.6	7.8	2.5
1953												
January	10	155	158	154	139	152	152	3.0	3.1	5.1	11.3	3.4
February	10	161	157	157	147	155	—	2.2	4.7	4.4	5.1	4.3
March	10	150	154	152	143	151	147	3.9	3.4	5.8	5.3	3.6
April	10	175	178	179	156	179	176	2.0	6.1	8.0	5.0	2.5
May	10	162	160	163	157	168	—	1.8	4.2	1.5	11.8	1.4
June	10	155	155	161	143	167	158	0.7	4.7	10.0	6.4	2.8

* Coleman method,[15] all others are Abell method.[14]

† Goldwater Memorial Hospital, F. Kendall and L. Abell.

‡ Framingham Heart Study, T. R. Dawber and J. Hill.

TABLE 6.—*Summary of Between-Laboratory Reproducibility Studies, January 1952–June 1953, S_f 12–20, Levels in mg./100 ml. Serum*

Date	No. of specimens measured in duplicate	Mean lipid values				Technical errors (σ_e)			
		Cleveland	Donner	Harvard	Pittsburgh	Cleveland	Donner	Harvard	Pittsburgh
1952									
January	10	41.6	47.9	43.7	42.8	2.8	4.0	3.4	3.7
February	10	37.7	38.4	39.2	37.0	3.3	4.9	3.5	5.4
March	10	35.4	44.6	39.5	42.1	3.2	4.8	3.7	6.5
April	10	31.8	39.8	37.0	37.8	4.4	3.2	5.1	6.2
June	10	27.8	34.0	29.2	28.3	3.2	6.5	2.0	3.7
July	9	28.3	32.8	29.3	30.7	4.0	2.1	3.0	3.2
September	10	50.9	53.0	46.4	45.4	3.8	2.3	5.0	3.2
October	10	47.8	52.8	44.4	42.4	5.2	5.7	4.4	5.3
December	10	42.0	50.2	44.5	48.5	3.8	3.3	7.5	4.6
1953									
January	10	25.4	33.2	25.6	27.0	4.3	3.5	2.0	4.5
February	10	33.0	39.0	37.8	39.2	3.3	4.2	4.5	5.5
March	10	29.4	31.8	35.8	28.6	3.9	3.2	4.8	4.0
April	10	32.0	36.4	29.6	35.7	3.8	3.6	4.7	2.7
May	10	34.4	35.2	32.0	30.8	2.8	4.0	4.0	4.3
June	10	28.0	34.6	23.2	31.4	3.5	4.3	6.5	4.2

plasma obtained from blood banks.* In each laboratory the samples were divided into coded duplicates and these were introduced into the routine work within a few days. This was done in a manner to prevent the operators identifying the test samples or determining the identity of pairs. It is believed that these between-laboratory reproducibility tests, the results of which are summarized in tables 5, 6, and 7, are representative of the performance of the several

* The material for 4 of these tests was supplied by Dr. R. C. Arnold of the National Heart Institute with the assistance of personnel at the U. S. Public Health Service Hospital at Staten Island.

TABLE 7.—*Summary of Between-Laboratory Reproducibility Studies, January 1952–June 1953, S_f 12–100, Levels in mg./100 ml. Serum*

Date	No. of specimens measured in duplicate	Mean lipid values				Technical errors (σ_e)			
		Cleveland	Donner	Harvard	Pittsburgh	Cleveland	Donner	Harvard	Pittsburgh
1952									
January.....	8	92.9	97.4	79.8	88.4	7.2	9.1	6.0	10.8
February....	10	70.5	70.8	70.8	79.0	6.4	7.2	9.2	10.4
March.....	10	65.0	73.2	59.8	64.6	12.3	7.8	6.8	11.5
April.....	10	83.6	83.0	74.1	84.1	12.7	10.6	6.5	11.2
June.....	9	53.3	64.0	54.0	53.6	10.9	5.4	8.0	12.8
July.....	9	50.8	57.2	42.4	50.9	6.3	4.7	5.9	5.3
September...	10	109.4	108.0	91.5	92.6	14.5	10.6	9.4	7.2
October.....	10	84.0	77.8	61.9	64.6	12.0	7.3	7.4	9.3
December....	9	107.5	120.2	102.1	113.2	7.5	7.9	9.9	12.5
1953									
January.....	10	40.0	47.0	28.7	36.2	7.7	5.8	4.7	5.3
February....	10	51.0	56.6	47.6	55.9	3.7	8.7	10.5	10.1
March.....	10	61.6	65.6	62.0	56.1	6.5	5.2	7.9	8.7
April.....	10	93.8	109.0	100.1	103.9	10.7	12.7	10.2	15.4
May.....	10	62.0	53.9	52.9	50.6	3.8	10.2	6.2	6.5
June.....	10	56.6	60.0	38.8	57.8	13.1	10.4	8.2	4.4

Note: Four specimens with mean values in excess of 300 mg. per cent excluded from these tabulations.

TABLE 8.—*Equivalence of Performance of the Analytical Ultracentrifuges as Demonstrated by Reference to a Calibrating Standard Cell, September, 1951*

Laboratory and machine	Value in equivalent mg./100 ml.	Per cent deviation from theoretic
Theoretical value...	866.0	—
Observed values:		
Cleveland		
Machine A.....	862.0	−0.5
Machine B.....	860.0	−0.7
Donner*.....	871.5	+1.0
Harvard		
Machine E I....	865.2	−0.1
Machine E II...	869.7	+0.4
Pittsburgh		
Machine I E....	860.2	−0.7
Machine II E...	854.0	−1.4

* Reported on 1 of 3 machines used in the study. The machines used at Donner were equipped with carefully selected optical components so that they had essentially the same physical constants.

laboratories. The technical error shown in these tables does not fully reflect the error due to secular fluctuation, that is, the long-term rise and fall of average measurement levels within each laboratory during the lifetime of the study. The σ_e for the various quantities are of similar magnitude for the various laboratories.

In the summer of 1951, Dr. E. G. Pickels of the Spinco Company invented a standard reference cell for calibration of the optical centrifuge. This cell and its use are described in greater detail in the Technical Symposium (loc. cit.). It consisted of a carefully calibrated and inscribed quartz wedge held in an appropriate container. When this was photographed in the rotor of the ultracentrifuge, an area on the image could be accurately related to a theoretic area computed from the combined cell and machine constants. A comparison of the performance of the various machines used in the study is shown in table 8. The greatest deviation of any machine was 1.4 per cent of the theoretic value and the other machines were always within 1 per cent of the computed value.

All these observations indicated that by January 1952 the laboratories were prepared to collect data for the evaluation of their objectives. The desirability of commencing the pooling of data only after these revisions and proofs of methodology had been completed was outweighed by certain practical necessities. All the laboratories had begun collection of data before January 1952, and in the instances of Cleveland and Donner the task was almost completed. The inclusive dates for collection of data by

TABLE 9.—*Groups Studied: Dates of Study and Procedures Used in Examination*

Laboratory and Group	Dates of study		Procedures used in examination					
	Start	Finish	History	Physical	Laboratory			Chest x-ray
					Urine	B. P.	ECG	
Cleveland								
White Motor Co.	12–50	12–52	+	0	+	+	0	0
Chrysler Motor Co.	9–50	8–53	+	+	+	+	+	+
Cleve. Graph. Bronze	5–51	10–51	+	+	+	+	0	0
General Electric Co.	2–51	7–53	+	+	+	+	+	+
Nickel Plate Railroad	10–51	5–53	+	+	+	+	+	+
Donner								
Framingham Heart Study	8–50	8–53	+	+	+	+	+	+
City of Los Angeles	8–50	12–52	+	+	+	+	+	+
Pan American Airways	6–50	3–53	+	+	+	+	+	+
United Airlines	7–50	3–51	+	+	+	+	+	+
Eastman Kodak Co.	11–50	12–51	+	+	+	+	+	0
Harvard								
Am. Mutual Ins. Co.	6–51	9–51	+	+	+	+	+	0
Dr. Burwell	11–51	5–53	+	+	+	+	+	0
Campbell Soup Co.	4–51	6–51	+	+	+	+	+	+
Dr. Chapman	7–51	1–53	+	+	+	+	+	0
Lahey Clinic	1–51	5–54	+	+	+	+	+	+
Metropolitan Life Ins. Co.	12–52	5–54	+	+	+	+	+	+
Mass. Inst. of Technology	2–52	5–54	+	+	+	+	+	+
Rexall Drug Co.	2–52	4–53	+	+	+	+	+	0
Standard Oil of N. J.	12–51	6–52	+	+	+	+	+	+
Swift and Co.	4–51	2–53	+	+	+	+	+	+
Oxford Diabetes Study	2–53	1–54	+	+	+	+	+	+
Pittsburgh								
Hoffman-LaRoche	5–51	6–53	+	+	+	+	+	+
Weirton Steel Co.	6–52	6–53	+	+	+	+	+	+
Federal Prisons	5–52	4–53	+	+	+	+	+	0
Westinghouse Electric Co.	4–52	10–52	+	+	+	0	0	0
Armco Steel Corp.	7–51	7–52	+	+	+	0	0	+
Ford Engineering Co.	2–53	5–53	+	+	+	+	+	+

+ Included in examination.
0 Not included in examination.

laboratory and population source are shown in table 9. The experience may be representative of the working conditions to which such methods are usually exposed, and to that extent reflect their performance.

For inclusion in the study it was required that each subject must have a measurement of S_f 12–20 with the value computed according to final calibration constants. It was agreed initially, however, that absence of a cholesterol measurement would not disqualify a subject from the study, and a number were admitted without a reported value for that measure. Thus it will be noted in subsequent sections that a cholesterol value was not available for 10 out of the 82 subjects who experienced new events.*

Clinical Evaluation

It was anticipated that the medical screening for admission to the study would be variable, since the

* Total cholesterol for 1 of the definite events from the Framingham Heart Study was not measured at Donner Laboratory when the lipoprotein measurements were made. A cholesterol measurement had been made at Framingham on an aliquot of the same specimen and this value has been used in this report. Although Framingham cholesterol measurements averaged 5–10 per cent lower than Donner's during that period, no adjustment has been made in the value reported by Framingham.

examinations were generally not arranged and were never conducted by the cooperating laboratories. A tabulation of the kind of physical examination is also shown in table 9. The medical departments of the sources were initially supplied with manifold forms (Forms 1) for the record of this examination (see Exhibit A). Form 1 was to be completed by the examining physician for subjects who appeared well, who gave a negative medical history for cardiovascular disease and whose electrocardiogram, urinalysis and blood pressure were normal. The blood pressure criteria were as follows: A reported blood pressure in excess of either 170 systolic or 100 diastolic was considered disqualifying.* A borderline blood pressure, i.e., above 145 systolic or 95 diastolic and not above 170 systolic or 100 diastolic, was disqualifying, in the absence of other significant cardiovascular disease, only if there was also reported some electrocardiographic abnormality (see Exhibit B for the definition of these abnormalities), or cardiac enlargement in any significant degree. Eyeground changes of Keith-Wagener classification II or greater[17] disqualified a subject as normal. The presence of urine protein or sugar in more than trace amounts disqualified and the presence of any of the following conditions disqualified a subject for inclusion as a normal: Diabetes mellitus; nephritis (except past history of pyelonephritis, nephrolithiasis, or loss of kidney); treatment with ACTH, cortisone, or related hormones; history of rheumatic heart disease; known congenital heart disease; syphilis or Buerger's disease.

The items on Form 1 that were required in order to qualify the form for acceptance in the statistical pool of data are also noted in Exhibit A. These criteria were intended to permit the inclusion of only those people who were free of signs of cardiovascular disease and of any known condition that would have permitted the prediction of a subsequent clinical event that might be identified with or confused with atherosclerotic disease.

The search for new events attributable to atherosclerosis consisted of a clinical re-examination of the subjects with the same facilities originally used. In most of the sources this was another routine examination.

At the onset of the study, it was assumed that a single year of follow-up would provide a sufficient number of new clinical events to permit evaluation of the various measurements. As the study progressed, it became clear that the goal of 10,000 subjects could not be reached within a reasonable time, and it was decided to lengthen the follow-up period to provide additional man-years of observation.

The Technical Group had agreed that a standard follow-up period should be pre-arranged for each source in order to prevent bias that might arise from a more extensive follow-up of any particular class of subjects Thus, it was agreed that follow-up for any source would be secured with local option at either 1 year ± 4 weeks, 18 months ± 5 weeks, or 2 years ± 6 weeks. In practice, it was found that there was considerable variability in the follow-up period among the various sources, and these follow-up limits were finally changed to 1 year ± 2 months, and 2 years ± 3 months. In addition, certain exceptions were made to conform with special local situations. All subjects originating in the Framingham and Los Angeles studies were accepted at the follow-up intervals arranged in those studies, generally 24 months for Framingham and 15 to 18 months for Los Angeles, but with many deviations. Follow-up for the Eastman Kodak subjects was available only for 1 date, July 1954, which meant that the follow-up period was variable in the range 31 to 44 months. In each of these sources, the follow-up interval was determined by the source without reference to lipid values. It is believed that bias was not introduced by the variations in this procedure. Finally, groups of subjects from certain sources were admitted with 1-year follow-up, although the majority of subjects in those sources had a 2-year follow-up. The sources where these exceptions were made were MIT, Metropolitan Life, and Lahey Clinic from Harvard and Weirton Steel from Pittsburgh. This was done in order to permit an earlier termination of the study than would have been possible with the 2-year follow-up. These sources conducted routine annual examinations so that the choice of follow-up period was available without bias.

At the time of follow-up, the examining staff was asked to complete a brief form (Form 2—see Exhibit A) that identified the individual and asked, "Has this person had any evidence of myocardial infarction, angina pectoris, or cerebrovascular accident since the date of the initial report?" The examiner was asked to answer this question, "no," "yes," "questionable," or "data unobtainable." This information served to identify the new events but also included many uncertain events. The laboratories then originated a more extensive investigation of each of these events.

There were 2 purposes in studying groups with stable employee rosters. This arrangement made large numbers of suitable subjects available to the study and also assured a maximum availability of these for follow-up. Secondly, such organizations seemed to minimize the introduction of bias into the clinical follow-up. In most instances, the physician in charge of the source population was supplied a carbon of the Form 1 (Exhibit A) after the lipid measurements had been completed and entered on this sheet. The lipid levels were thus known to the

* Ordinarily only 1 blood pressure was reported on Form 1. Where more than 1 was reported, the classification was based on (a) the lowest, if not additionally described, or (b) the resting pressure, or (c) the left arm pressure, or (d) the seated pressure.

clinical personnel during most of the follow-up period. However, all the lipid data were filed with the statistician before the follow-up information was reported to the laboratory on Form 2. The integration of the measurements of the Cooperative Study with the existing program of clinical management of the groups seems to be the best assurance that the follow-up was applied to all the subjects in an unbiased manner.

The entire study turned upon the identification of a sufficient number of individuals in the study population who were well when first measured by the criteria outlined above and who then developed new evidence of clinical disease that could reasonably be considered a consequence of atherosclerosis. These clinical events were primarily reflections of coronary heart disease but included other manifestations of cardiovascular disease. They were composed of signs and symptoms supporting a diagnosis of angina pectoris, coronary thrombosis, myocardial infarction, peripheral vascular disease (other than Buerger's disease), cerebrovascular accident, and "sudden deaths." Information was obtained for subjects who died with congestive failure, rheumatic heart disease, and pulmonary embolism although these were not utilized in the present evaluations. Death certificate information was also sought for subjects believed to have died of noncardiovascular causes.

TABLE 10.—*Categories of New Events, Classification by the Review Committee. Men, 40–59*

Definite Events
1. Myocardial infarction, definite
2. Myocardial infarction, definite, by ECG only
3. Coronary thrombosis, definite
4. Coronary sclerosis, definite, by autopsy
5. ECG abnormality, definite, assoc. with coronary artery disease
6. Angina pectoris, definite, with ECG changes
7. Angina pectoris, definite, w/o ECG changes
Probable or Possible Events
8. Myocardial infarction, probable
9. Coronary thrombosis, probable
10. ECG abnormality, probable, assoc. with coronary insufficiency
11. Angina pectoris, probable, w/o ECG changes
12. Myocardial infarction, possible, by ECG only
13. Coronary thrombosis, possible
14. ECG abnormality, possible, assoc. with CAD
15. Angina pectoris, possible

A questionnaire, called Form 5, was supplied to the examining staff or the physician who attended the patient. This asked for the identification and documentation of the nature of the new event identified on the Form 2. The Form 5 consisted of 4 major sections for use with instances of angina pectoris, myocardial infarction, cerebrovascular accident, and other significant cardiovascular disease. Appropriate questions were proposed under each of these sections. All the solicited information was *apropos* the date of the follow-up examination described in the Form 2.

Instances of sudden death posed a special problem for there are often no definitive clinical findings which will identify the cause. In order of preference, the following documentation was sought for these: an autopsy protocol, a clinical summary of the attending physician, and a death certificate.

Review Committee

It was important that the classification of individuals who had developed new evidences of atherosclerosis be done in an unbiased manner. Dr. Paul D. White agreed to assist the Technical Group with this task and he arranged for Dr. Samuel A. Levine and Dr. Howard B. Sprague to serve with him as a review committee. The members of this committee reviewed the evidence that was supplied for each subject through the statistical consultant and determined whether there was justification for classifying the subject as "normal" at the time of admission to the study and then, whether the available evidence supported the clinical impression of "new disease." Finally they recorded their collective opinion of the nature of this new event. The Review Committee sometimes deferred judgment and sought additional evidence either through additional efforts of the laboratory or with their personal letters to the attending physicians. The Review Committee was not told the lipoprotein or cholesterol values, nor the identity of the laboratory that had processed each subject, although the identity of these could have been deduced in some instances from the place at which the illness had occurred.

The Review Committee classified all "new events" according to the plan shown in table 10. It should be emphasized that the "new events" were identified as of the time of re-examination of the entire group of which the subject was a part. Certain subjects were classified with the

assistance of clinical information that appeared after the follow-up interval but later information was used as supplementary evidence and not as grounds for inclusion for consideration among the "new events."

The several kinds of diagnoses that were assigned to these new events by the Review Committee have been divided subsequently into 2 categories according to the certainty of the diagnosis expressed by the reviewers. This division, which was made by the research group, assigns to the category termed "definite new events" the 7 classifications of coronary artery disease for which a definite diagnosis could be established. This group includes 65 new events. The "probable or possible new events" comprise the 17 new events for which either the paucity of information or uncertainty of its interpretation makes the diagnosis less certain.

Evaluation of Bias

At a number of points in this report, references have been made to precautions that were taken to avoid bias. These included the provisions that laboratory results be reported to the statistical office in advance of the collection of follow-up data from the source, and that all reported "new events" be evaluated independently by a committee of clinicians who were unaware of the lipid measurements at entry to the study. One result of this evaluation was the exclusion by the Committee of 24 subjects with reported new events on the grounds that they were incorrectly classified as normal at entry. This reclassification was based on a review of serial electrocardiograms and of clinical history, including history of hospitalization. By prearrangement, knowledge of lipid values was withheld from the Review Committee. The data for each of these 24 cases are shown in Exhibit F. The clinical status of those subjects for whom no new event was reported was not subject to a similar review.

Another source of possible bias is differential follow-up. Eighty per cent of the candidates for follow-up were re-examined at the specified follow-up interval. About 12 per cent were followed at intervals outside the established limits and were excluded from consideration. An additional 7.5 per cent were not re-examined. This number of subjects who were not followed would permit the possibility of bias.

The lipid values were made available to the medical personnel of the originating source prior to the follow-up although some sources elected not to receive these data. Under these circumstances it is conceivable that a source might have been more energetic in tracing persons with high lipid values, or that a laboratory might have stimulated a special effort in the follow-up of persons with high lipid values. These activities seem most unlikely. There is indirect evidence bearing on this matter in the lipid values for the groups that were either not followed or were followed but at other than a standard follow-up interval. Judging from the data in Exhibit C there is little difference in mean lipid values between the "followed and pooled" group and the remainder who qualified at entry but were excluded by follow-up limitations. This evidence is not a proof of the absence of bias, but it seems to minimize that possibility.

Statistical Methods

The 2 principal objectives of the study can be restated in forms more appropriate to statistical testing and generalized to other lipid measures in these hypotheses: 1. If a group of presumably normal men is observed for a specified period of time subsequent to the measurement of their lipid values, a subgroup will be found to have developed clinical evidence of significant atherosclerotic disease, and that subgroup will be found to have had lipid values that were high initially relative to the distribution of lipid values in the total group. 2. In the subgroup developing new disease, values of lipid measure A will be found to have been higher (or lower), relative to the distribution of measurements of A in the total group, than were the values of lipid measure B, relative to the distribution of measurements of B in the total group (where A and B may be any of the several lipid measures). These 2 hypotheses formed the central core of the Cooperative Study.

The evaluation of these hypotheses was based upon the concept of a "base population" out of which the "new event" cases could be assumed to have arisen as a subgroup. This base popula-

tion was the group of presumably normal men (according to the criteria of the study) aged 40 to 59 at entry to the study for whom a prearranged follow-up observation was completed. The "new events" were the occurrences in individuals in this base population of significant atherosclerotic disease in the follow-up period. Only 1 event per individual is counted. The "definite new events" are those new events with sufficient clinical evidence that the Review Committee regarded them as unequivocal.

At the outset of the study, it was assumed that the performance of the various lipid measurements could be carried out with a high degree of accuracy and reproducibility. This would have permitted pooling of the data in the simplest fashion, that is, by treating the data from all laboratories as a single, homogeneous lot. It was soon found, however, that there were 2 sources of variation in the lipid data that indicated that these could not be treated in this manner. There were technical variations between laboratories, and there were variations of population values among certain important source groups.

The technical variation between laboratories has been discussed in preceding sections and summarized in tables 5, 6, and 7. In view of the between-laboratory differences in mean values found in the reproducibility studies, the following procedure has been used for pooling results. For each of the lipid measures a separate population base was formed for each laboratory by simple pooling of the data for the sources within the laboratory and the lipid values for the new events were referred to the laboratory population base.

There were 2 exceptions to this procedure. The Los Angeles civil service population was treated as a separate group and the lipid values for new events in the Los Angeles group referred to the Los Angeles population values. The prisoner population was excluded from the Pittsburgh base, and the 2 prisoner new events referred to the remaining Pittsburgh population base. These exceptions were based on the following considerations: The mean values and standard deviations for 19 sources or source groups as shown in table 11 were examined for homogeneity. Two groups appeared to be markedly different from others. The Los Angeles city employees showed a mean cholesterol level remarkably higher than that of any other group studied, and a pattern of S_f 12–20 and S_f 20–100 measurements also at variance with those of the other groups studied by the Donner Laboratory. In an earlier analysis of these data,[18] Donner had considered this difference in lipoprotein patterns in the Los Angeles source and attributed it to the fact that the blood was drawn in the fasting state. Although there is no independent evidence that fasting would bring about changes of the observed kind and magnitude in either lipoprotein or cholesterol levels, it seemed desirable to treat the Los Angeles group as a separate entity, and this has been done with respect to all of the lipid measures. The prisoner group studied in Pittsburgh differed from other groups studied by that laboratory in having remarkably low values for cholesterol and S_f 12–20. It seemed reasonable to assume that peculiarities of diet, or mode of life generally, might have brought about differences in mean lipid values for this group, although there is no definite evidence for this. With acceptance of this assumption, it was decided that data for the prisoner group should not be used in the computation of values for the base population against which new events were to be compared, and that new events originating in the prisoner group should be evaluated with the base population used for assessment of all other new events occurring in the Pittsburgh sources. The prisoner group contributed only 2 definite new events and because of the relatively small number of subjects in the prisoner base population, the net effect of this special handling of the prisoner population is, in fact, trivial.

There are available several alternative methods for comparing the values of lipid measures in the new events with those in the base populations out of which they arose. The 2 chosen were a comparison based on mean differences and a comparison of the percentile ranking of individuals by the several measures.

In comparing the mean value of a lipid measure in a subgroup of new events with that observed in the population out of which the subgroup was drawn, the following null hypoth-

TABLE 11.—*Mean Lipid Values and Standard Deviations by Source, Men. 40–59, Levels in mg./100 ml. Serum*

Laboratory and source	Number of men*			Mean values			Standard deviations		
	S_f 12–20	S_f 20–100	Cholesterol	S_f 12–20	S_f 20–100	Cholesterol	S_f 12–20	S_f 20–100	Cholesterol
Cleveland									
White Motor Co.	235	230	—	32.5	72.7	—	18.4	62.5	—
Chrysler Motor Co.	464	460	196	33.3	76.6	237.0	24.9	58.9	49.9
Cleve. Graphite Bronze	264	264	65	36.9	101.1	246.3	23.0	74.3	55.5
General Electric Co.	183	181	151	34.7	84.2	247.1	17.6	57.4	48.2
Nickel Plate Railroad	63	63	63	29.1	71.2	236.6	14.7	45.3	47.7
Donner									
Framingham Heart Study	837	837	703	47.3	114.9	240.0	21.7	86.1	44.8
City of Los Angeles	686	686	541	55.8	84.4	263.1	26.2	60.4	57.7
Pan American Airways	130	130	100	47.7	91.3	243.4	25.8	67.7	46.8
United Airlines	64	64	33	48.6	102.6	256.1	21.9	63.4	42.8
Eastman Kodak Co.	371	371	327	51.2	99.7	242.0	25.7	54.5	51.0
Harvard									
Lahey Clinic	136	135	111	40.1	84.3	252.5	22.3	63.5	49.6
Metropolitan Life Ins. Co.	534	534	533	38.9	85.7	240.7	18.9	59.9	44.7
Mass. Inst. of Technology	76	76	76	40.8	78.5	244.4	19.4	61.9	49.0
All others†	97	97	79	40.1	87.3	240.9	25.4	55.0	46.7
Pittsburgh									
Hoffman-LaRoche	201	199	199	44.2	91.5	224.3	21.1	62.1	42.8
Weirton Steel Co.	367	367	367	46.4	103.8	220.2	25.4	85.6	42.1
U. S. Penitentiaries: Prisoners	236	236	236	36.1	96.3	194.6	18.8	59.8	36.4
U. S. Penitentiaries: Staff	158	158	158	41.7	107.6	209.0	25.9	71.0	40.9
All others†	47	45	47	48.0	112.3	212.7	29.9	75.4	46.0

* Includes only men with completed follow-up who were normal at entry to the study.

† Sources with less than 50 men are not shown separately but are included in the residual group for the laboratory.

esis was used: the mean lipid level in the individuals experiencing new events in comparison with the mean level in the base population out of which they arose, is no higher than would be expected by sampling variation in successive subsamples from such a parent population. This hypothesis was evaluated by the statistic t which has the distribution of a normal deviate.

$$t = \frac{\bar{D}}{\sigma/\sqrt{n}}$$

where $\bar{D}$ = mean lipid level of new events minus mean lipid level of base population, σ = standard deviation of the lipid measure for base population, n = number of new events.

The 1-sided nature of the hypothesis being tested requires that the probability be evaluated in terms of a single tail of the normal distribution. The combination of differences from laboratories with both technical differences of measurement and population differences poses a new problem, since the number of observations contributed by each laboratory becomes important. If it is assumed that the laboratory means differ, but the distributions have a common variance, the new events are referred to the mean for each laboratory and the test takes the alternate form:

$$t = \frac{1/n\sum n_i \bar{D}_i}{\sigma/\sqrt{n}}$$

Here $\bar{D}_i$ is the difference within a laboratory between the mean lipid value for new events and the mean lipid value in the base population. The number of new events in the given laboratory is n_i, and $1/n\ \Sigma n_i\ \bar{D}_i$ is, therefore, the weighted mean of the mean differences. In this case σ is a pooled estimate of the standard deviation.

In comparisons based on percentiles, each subject has been assigned a percentile score for each lipid measure in the distribution of the

values of that measure for the base population of which he was a member.

If an arbitrary point is established in the distribution of a given base population, e.g., the fiftieth percentile point, or the seventy-fifth percentile point, and a count made of the number of new events with a percentile score for any given lipid measure above that cutting point, it is possible to test the null hypothesis that the proportion of new events with percentile scores above the cutting point is no greater than would be expected by sampling variation in successive subsamples from such a parent population. For cutting points at the fiftieth and seventy-fifth percentiles, this may be evaluated by reference to the binomial distribution and, again, in this type of 1-sided hypothesis, by a single tail of the distribution.

Neither of these procedures can be used to test directly, with respect to any 2 lipid measures, which of the 2 was superior in predicting new events in the population by characterizing those individuals with a high value. One method of accomplishing this is to compare the percentile scores with which 2 lipid measures characterize the same individual who later developed a new event. This has been tested in terms of the null hypothesis that the mean difference between the percentile scores of 2 lipid measures for a group of individuals with new events is no greater than would be expected by chance with such a distribution of individual differences. This is evaluated in terms of the statistic t, which will be distributed as Student's t. (In view of the previous use of 1-sided tests, it should be noted that this is a 2-sided test.)

$$t = \frac{\bar{d}}{\sigma_{\bar{d}}}$$

where d = difference between the percentile scores on 2 lipid measures for a given individual, $\bar{d} = \Sigma d/n$, $\sigma_{\bar{d}} = \sqrt{\Sigma (d - \bar{d})^2/n(n-1)}$, and n = number of new events.

This test is sensitive to differences in position over the entire range. Alternatively, it is possible to compare the performance of 2 measures in placing an individual above or below some arbitrary cutting point, e.g., the fiftieth or seventy-fifth percentile. That measure is considered "better" which, when the 2 measures disagree, places a significantly larger proportion of the new events at the upper end of the percentile scale. A placement above the cutting point may be considered a "success," and 1 measure may succeed at this when another fails, and vice versa. Without regard to the number of times that the 2 measures agree, the consideration is only of those instances where they disagree. If measure A succeeds in a cases where measure B fails, and measure B succeeds in b cases where measure A fails, then the test is whether the ratio a/(a+b) differs significantly from one half. (This is also a 2-sided test.)

Two other methodologic points deserve special attention: asymmetry of the distributions and age adjustment. The frequency distribution of the total cholesterol, S_f 12–20 and S_f 20–100 levels for the individual laboratory sources are shown in figures 1, 2, and 3. These distributions show varying degrees of asymmetry or skewness, that for cholesterol showing less skewness than the other 2. The statistical tests that are used in this report are relatively insensitive to departures from normality not greater than those encountered in these distributions, and accordingly the original data have been used without transformation.*

The necessity of age adjustment of study groups with respect to serum lipid levels was also considered. It has been contended[19] that serum cholesterol levels vary with age although the basic data used in that study were unique in respect to methodology and study population. The data also required excessive interpolation in order to establish the age level relationship. Jones and co-workers[20] have described the relation of lipoprotein levels and age. They observed a large increase of the S_f 12-20 level in men, particularly in the third decade of life. The levels were then nearly constant through age 60. Those authors found that the serum cholesterol increased slowly but perceptibly in

* Various transformations were tested; the square root and logarithmic appeared to be most successful. Parallel analyses, using the logarithmic transformation, indicated that conclusions would be substantially the same as those reached here with untransformed data.

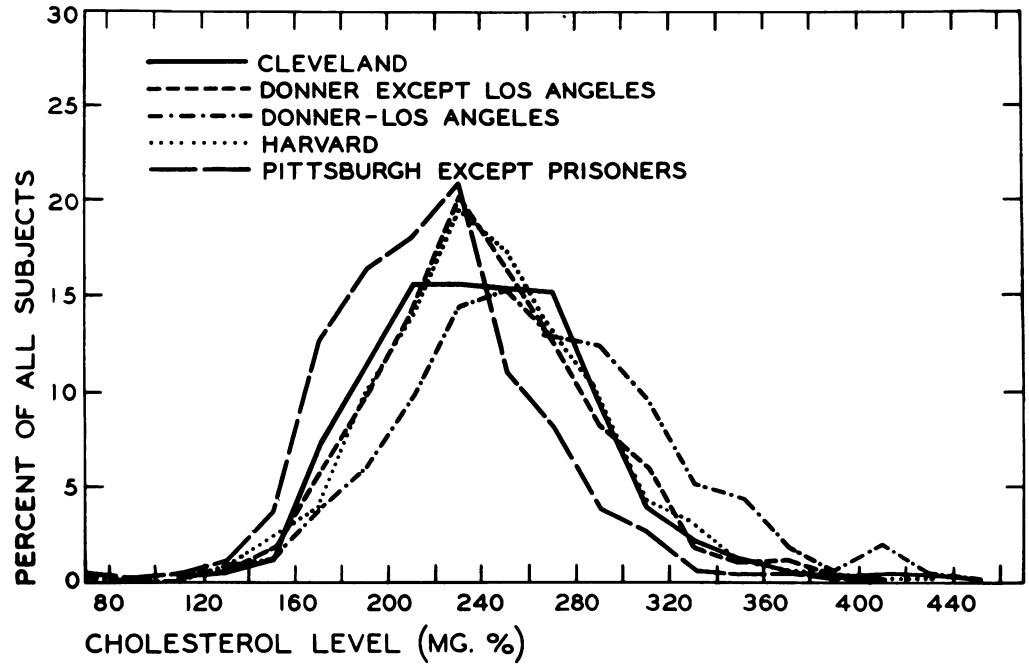

FIG. 1. Distribution of all subjects by cholesterol level, men 40–59.

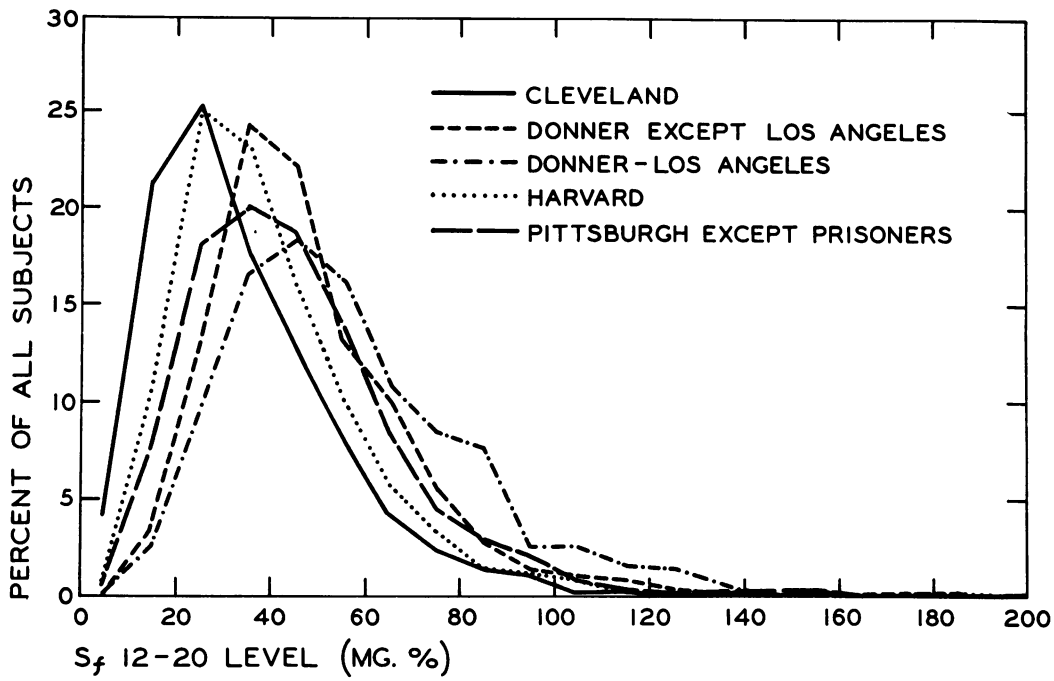

FIG. 2. Distribution of all subjects by S_f 12–20 level, men 40–59.

men throughout the age span 25–65 years. In another publication from the Donner Laboratory[18] the relation to age of the "standard" lipoprotein levels, i.e., measurements adjusted for the influence of concentration upon the ultracentrifugal measurement (*v.s.*) was described. The lipoprotein bands pertinent to this discussion, i.e., S_f^0 12–20 and S_f^0 20–100,

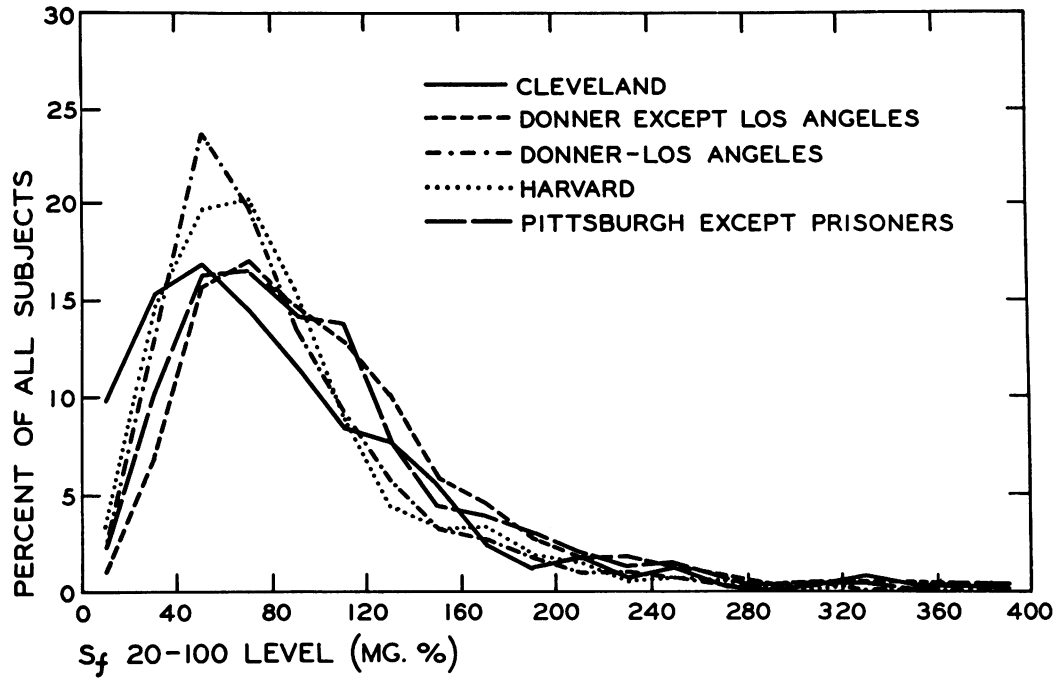

FIG. 3. Distribution of all subjects by S_f 20–100 level, men 40–59.

TABLE 12.—*Mean Lipid Values by Age, Men, 40–59, Levels in mg./100 ml. Serum*

Measure and age	Cleveland	Harvard	Pittsburgh (except prisoners)	Donner	
				except Los Angeles	Los Angeles
S_f 12–20					
40–44	36	38	45	48	53
45–49	34	41	46	49	57
50–54	33	39	44	52	56
55–59	33	40	44	45	59
S_f 20–100					
40–44	94	83	102	109	88
45–49	82	88	111	110	82
50–54	78	84	94	113	79
55–59	77	84	93	97	88
Cholesterol					
40–44	242	236	213	237	258
45–49	241	248	223	242	260
50–54	244	243	219	248	268
55–59	240	247	223	241	269

Note: Includes only men with completed follow-up who were normal at entry to the study.
No mean is based on less than 96 subjects.

showed a curvilinear relationship with age for the men forming a smooth parabola with the maximum at about age 55. All these studies seemed to indicate a small but real change of cholesterol and lipoprotein level with age for men after 30 years of age.

The data from each of the laboratories were examined for the change of serum lipid level within the 40–59 year age group. The cholesterol, S_f 12–20 and S_f 20–100 levels with age are shown for the 4 laboratories (and Los Angeles) in table 12. The small differences observed in this age range indicated that it was unnecessary for the purpose of the ensuing analysis to consider age effect.

RESULTS

The base population of 4,914 men yielded 82 men who developed clinical manifestations of disease that were accepted by the Review Committee as caused by atherosclerosis.* A description of the 82 new events is shown in table 13. Sixty-five of the new events were

* Not included in these figures are the 7 new events arising out of the group of 508 White Motor Co. employees studied by Cleveland. These new events were reported without the complete documentation required for Review Committee action and are therefore not strictly comparable to the 82 events from other sources that were documented and reviewed. The characteristics of the individuals experiencing these new events are shown in Exhibit E.

TABLE 13.—*Number of New Events by Category of Event, As Classified by the Review Committee, Men, 40–59*

	All sources	Cleve.	Donner		Harvard	Pitts.
			except L.A.	L.A.		
Totals	82	15	22	14	12	19
Definite events	*65*	*12*	*18*	*12*	*8*	*15*
Myocardial infarction definite	38	10*	8	6	5	9
Myocardial infarction, definite, by ECG only	3	—	2	—	—	1
Coronary thrombosis, definite	6	1	4	—	—	1
Coronary sclerosis, definite by autopsy	1	—	—	1	—	—
ECG abnormality, definite, assoc. with coronary artery artery disease	6	—	2	—	2	2
Angina pectoris, definite, with ECG changes	4	—	2	1	—	1
Angina pectoris, definite, w/o ECG changes	7	1	—	4	1	1
Probable or possible events	*17*	*3*	*4*	*2*	*4*	*4*
Myocardial infarction, probable	1	—	—	—	—	1
Coronary thombosis, probable	4	3	—	—	—	1
ECG abnormality, probable, assoc. with coronary insufficiency	1	—	1	—	—	—
Angina pectoris, probable, w/o ECG changes	4	—	—	1	2	1
Myocardial infarction, possible, by ECG only	1	—	—	1	—	—
Coronary thrombosis, possible	1	—	—	—	1	—
ECG abnormality, possible, assoc. with CAD	1	—	1	—	—	—
Angina pectoris, possible	4	—	2	—	1	1

* Does not include 2 White Motor cases.

classified as "definite events." It is on these "definite new events" that most of the subsequent analysis will be based.

The data obtained for the base populations can be shown graphically in cumulative form as the proportion of subjects (ordinate) with lipid values below any given level (abscissa). These S-shaped curves facilitate evaluation of measurements of new events by indicating the position of these with reference to the cumulative distribution of the appropriate base population. For the present purposes cumulative curves have been presented for the distribution of each lipid measure in the base population studied by each laboratory. (The Donner Laboratory data have been presented in 2 parts—Donner exclusive of the Los Angeles city employee group, and the Los Angeles city employee group.) Each definite event has been located on its appropriate distribution curve according to the lipid measurement it showed. If an elevation of a lipid measurement does permit the prediction of the development of clinically manifest atherosclerotic disease, it follows that the subjects experiencing definite new events should have lipid levels that are concentrated in the upper part of the distribution curve of the base population. An impression of the extent to which this is true can be gained from the graphic presentations; the extent to which it is statistically significant can be evaluated by the application of the various tests described in the preceding section.

In figure 4 the cholesterol levels of the definite events are shown in this manner. Figures 5 and 6 show the corresponding distributions for S_f 12–20 and S_f 20–100 measures. It is apparent that none of these measures has led to a clear separation of the definite new events at the high levels, and indeed none has regularly placed the individuals in the upper half of the distribution.

The success of any lipid measure in associating a high level with subsequent development of atherosclerotic disease can formally be tested in terms of a specified value, or cutting point, above which the definite new events

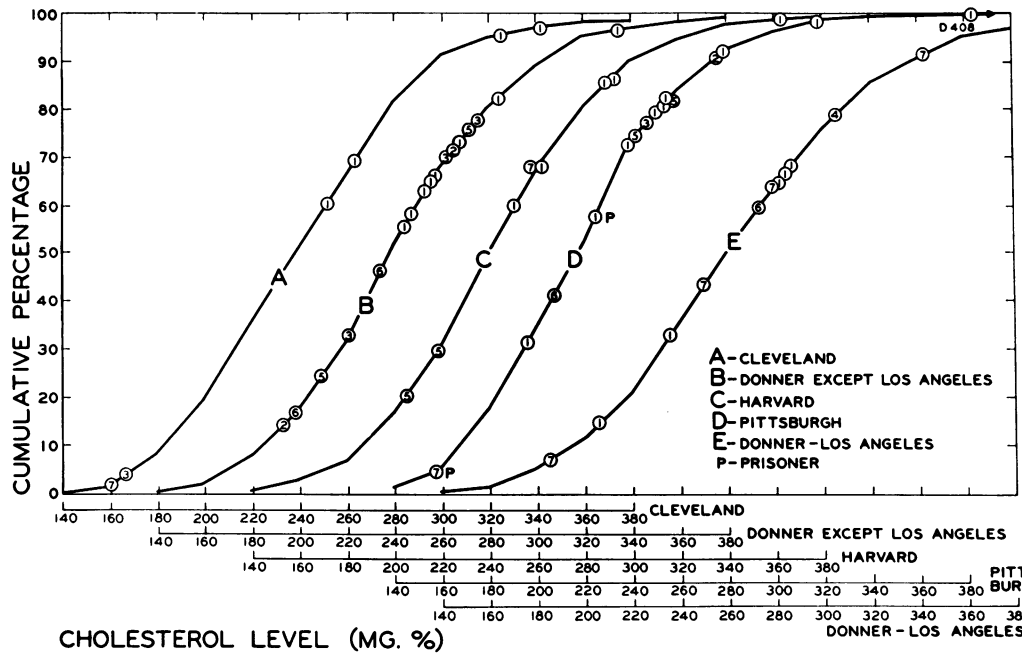

FIG. 4. Definite new events related to cumulative percentage of all subjects according to cholesterol level, men 40–59. To find the proportion of the base population having lipid levels below a specified amount, find the lipid level on the appropriate abscissa scale, read up to the intercept on the curve for the base population and read over to the corresponding percentage point on the ordinate scale. The cumulative percentage curves for the base populations are plotted with a common scale for cumulative percentage but with differing scales for lipid levels. Thus, 50 per cent of the Harvard base population are seen to have cholesterol levels below 240.

Percentile values for the definite new events (as given in Exhibit D) were calculated from the exact distribution rather than graphically. In some instances this results in a minor discrepancy in graphing. In such cases, the point is plotted at the exact percentile position, even though the lipid level indicated by the curve at that point may differ slightly from the exact value.

The numbers within the circles refer to the diagnostic categories listed in table 10.

might be expected to fall. There seems to be no *a priori* basis for the selection of any specific cutting point for a crucial test in the present problem. Lacking this, data are presented in table 14 using both the fiftieth percentile (the median) and the seventy-fifth percentile as cutting points. It should be noted that these points are arbitrary, and that different conclusions might be reached by comparisons at other cutting points.

The cholesterol measurements of all laboratories combined showed a highly significant* ability to place the subjects with definite events above the fiftieth percentile. The S_f 12–20 measure did not show a significant ability to accomplish this separation; the S_f 20–100 measure showed significant separation at the fiftieth percentile. On the combined data, none of the measures showed significant separation at the seventy-fifth percentile.

Another way of examining the predictive ability of a lipid measure is to compare the mean value of the measure in the group of new events with the mean value in the base population. The mean differences for the 3 lipid measures are shown in table 15. When the combined experience of all the laboratories is examined, it will be seen that all of the mean differences are positive, i.e., the mean value of each of the lipid measures was higher in the new events than in the base

* In the ensuing discussion the arbitrary but somewhat useful convention is used of referring to statistical significance at the 5 per cent level as "significant" and at the 1 per cent level as "highly significant."

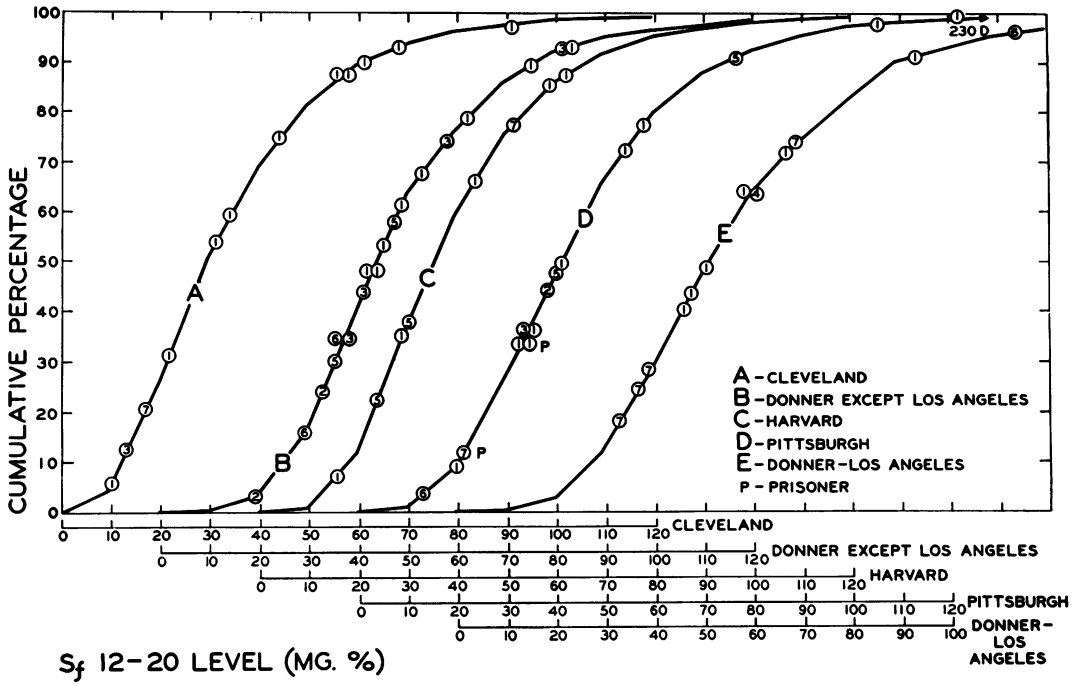

FIG. 5. Definite new events related to cumulative percentage of all subjects according to S_f 12–20 level, men 40–59.

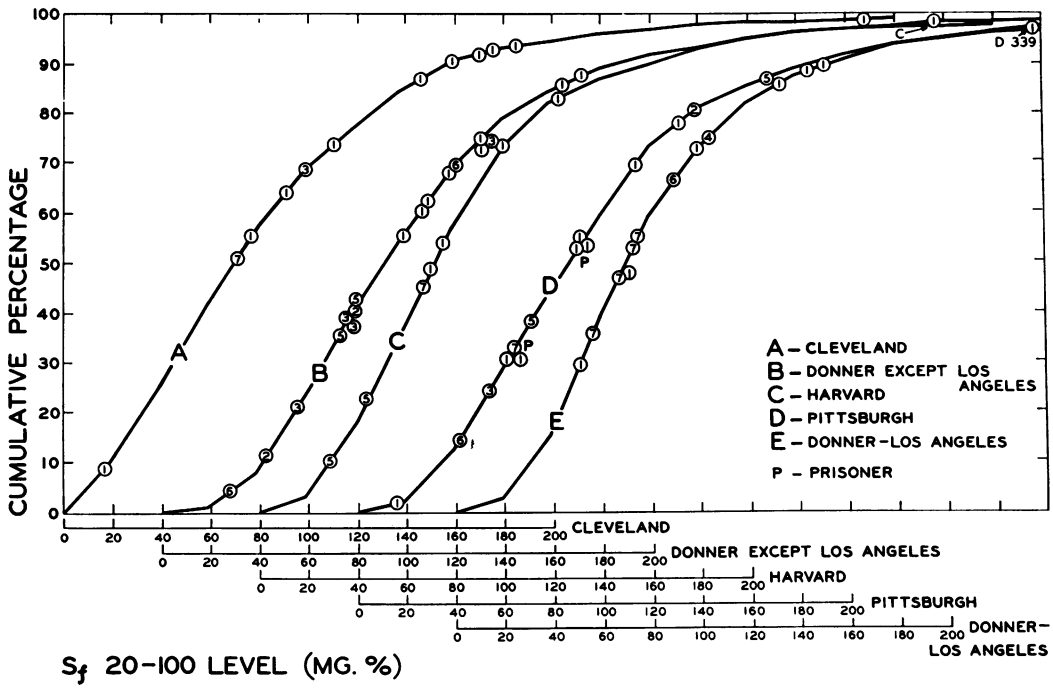

FIG. 6. Definite new events related to cumulative percentage of all subjects according to S_f 20–100 level, men 40–59.

TABLE 14.—*Separation of Definite New Events at Specified Percentile Cutting Points, Men, 40–59*

Measure and source	Number of new events		
	Total	Above fiftieth percentile	Above seventy-fifth percentile
S_f 12–20—All sources	*65*	*32*	*18*
Cleveland	12	8	5
Donner, except Los Angeles	18	9	4
Donner, Los Angeles	12	6	2
Harvard	8	4	3
Pittsburgh	15	5	4
S_f 20–100—All sources	*65*	*42**	*18*
Cleveland	12	11	6
Donner, except Los Angeles	18	10	2
Donner, Los Angeles	12	9	4
Harvard	8	4	2
Pittsburgh	15	8	4
Cholesterol—All sources	*57*	*41†*	*20*
Cleveland	6	4	2
Donner, except Los Angeles	17	12	4
Donner, Los Angeles	11	7	2
Harvard	8	6	3
Pittsburgh	15	12	9

Significance at a 5 per cent level denoted by * at a 1 per cent level by †. Test based on the binomial distribution and expresses the probability of at least the number of events reported above the specified percentile. The number of new events from individual laboratories is generally too small to expect significance considering the variability among new events, and tests for individual laboratories are therefore omitted.

Other measurements made only by the Donner Laboratory and referred to by that laboratory as "standard" values will be found in Discussion A.

TABLE 15.—*Mean Deviation in Lipid Level for Definite New Events From Levels for Base Population, Men, 40–59*

Measure and source	New events		Base population: Mean	Mean difference: New events — base
	Number	Mean		
S_f 12–20—All sources	*65*	—	—	*4.5*
Cleveland	12	42.0	34.3	7.7
Donner, except Los Angeles	18	48.4	48.4	0.0
Donner, Los Angeles	12	59.3	55.8	3.5
Harvard	8	39.5	39.4	0.1
Pittsburgh	15	55.5	45.0	10.5
S_f 20–100—All Sources	*65*	—	—	*10.3*
Cleveland	12	135.5	84.4	51.1
Donner, except Los Angeles	18	99.7	103.1	−8.4
Donner, Los Angeles	12	92.6	84.4	8.2
Harvard	8	97.8	85.0	12.8
Pittsburgh	15	102.3	101.9	0.4
Cholesterol—All sources	*57*	—	—	*17.4†*
Cleveland	6	251.7	241.4	10.3
Donner, except Los Angeles	17	251.0	241.3	9.7
Donner, Los Angeles	11	266.5	263.1	3.4
Harvard	8	267.4	242.7	24.7
Pittsburgh	15	254.0	218.5	35.5

Significance at a 1 per cent level denoted by †. Test based on normal distribution and expresses the probability of a positive mean difference. The number of new events from individual laboratories is generally too small to justify the assumption of normality considering the underlying distributions. Mean differences for individual laboratories have therefore not been tested.

Other measurements made only by the Donner Laboratory and referred to by that laboratory as "standard" values will be found in Discussion A.

population. The difference for cholesterol was highly significant, but the small differences observed for S_f 12–20 and S_f 20–100 were not significant.

The fourth objective of the Cooperative Study was "to compare total cholesterol and S_f 12–20 fractions as indicators of the disorder."

The relative predictive ability of 2 lipid measures may be assessed by comparison of these at various cutting points or by a comparison of percentile scores over the entire range. The selection of a cutting point is an arbitrary matter that gives great weight to small differences that bring about disagreement between 2 measures in the neighborhood of the cutting point, and no weight to larger differences of the measures that do not cross a cutting point. Comparisons of lipid measures (cholesterol and S_f 12–20, cholesterol and S_f 20–100, S_f 12–20 and S_f 20–100), using the fiftieth and seventy-fifth percentiles as cutting points, are shown in table 16. All of the measures agree more often than they disagree. In those subjects where 2 measures disagree, there is 1 highly significant difference, namely the

TABLE 16.—*Comparisons of Specified Lipid Measures Based on Placement of Definite New Events Relative to Fiftieth and Seventy-fifth Percentiles, Men, 40–59*

Measure A	Measure B	Agreement: Both measures on same side of cutting point			Disagreement: One measure above, the other below cutting point		
		Total	Both above	Both below	Total	A above	B above
Seventy-fifth percentile							
Cholesterol	S_f 12–20	34	7	27	23	13	10
Cholesterol	S_f 20–100	37	7	30	20	13	7
S_f 12–20	S_f 20–100	49	10	39	16	8	8
Fiftieth percentile							
Cholesterol	S_f 12–20	38	24	14	19	17†	2
Cholesterol	S_f 20–100	35	27	8	22	14	8
S_f 12–20	S_f 20–100	41	25	16	24	7	17

If one measure discriminates better than another at a 1 per cent level, this is denoted by †.

If a measure is above the specified percentile, this may be considered as a "success" for that measurement. Where both measures for a definite new event "succeed" or both "fail" the measures may be considered as equally effective. To determine whether 1 measure is more effective than the other, therefore, we consider only these instances where 1 succeeds while the other fails and test whether either measure is favored more than half the time.

Other measurements made only by the Donner Laboratory and referred to by that laboratory as "standard" values will be found in Discussion A.

advantage of cholesterol over S_f 12–20 at the fiftieth percentile cutting point. None of the other comparisons shows significant differences between measures.

Another way of evaluating the relative capacity of 2 lipid measures to predict the new events within a base population is to compare the percentile scores that the 2 measures assign the same individual. This method credits separation at all levels. Table 17 shows the differences in mean percentile scores for the following comparisons: cholesterol and S_f 12–20, cholesterol and S_f 20–100, S_f 12–20 and S_f 20–100. The only difference that is significant is the higher mean percentile value of cholesterol when this is compared with S_f 12–20.

It should be emphasized that these various tests presented in tables 14 through 17 are not independent, all being related ways of looking at the same data. Taken as a group they suggest that cholesterol was the most effective measure in separating out the definite new events, and S_f 12–20 the least effective.

There were 17 subjects who developed signs of new disease which the Review Committee agreed were indicative of the presence of atherosclerotic disease, but which they could not classify as "definite." These events have been classified as "probable" or "possible" new events and their identity and individual measurements are tabulated in Exhibit D. The lipid levels of these subjects are on the average very similar to the levels found in the base population.

TABLE 17.—*Comparisons of Specified Lipid Measures Based on Percentile Position of Definite New Events: Men, 40–59*

Lipid measures compared		Number of events (N)	Mean percentile score		Mean difference A-B
Measure A	Measure B		Measure A	Measure B	
Cholesterol	S_f 12–20	57	61.3	53.2	8.1*
Cholesterol	S_f 20–100	57	61.3	55.4	5.9
S_f 12–20	S_f 20–100	65	53.8	57.6	−3.8

Significance at a 5 per cent level denoted by *. Test based on a 2-sided t distribution with N-1 d.f.

Other measurements made only by the Donner Laboratory and referred to by that laboratory as "standard" values will be found in Discussion A.

DISCUSSION

*Note by Referee**

The participants in this study concur in the presentation to this point. They agree in find-

* Dr. E. Cowles Andrus agreed to act for the Committee on Lipoproteins and Atherosclerosis and the

ing that atherosclerosis, as manifested by clinical signs of coronary artery disease, is associated with a disorder of lipid metabolism and that there is some predictive value in the various lipid measurements examined. However, because of clear divergence of opinion between the Eastern Laboratories and the Donner group with regard to the degree and specificity of this predictive value, and indeed with regard to the significance of certain data in relation thereto, it was agreed that the discussion and conclusions would be prepared independently and presented separately by 2 groups: Dr. Gofman and his colleagues at the Donner Laboratory to present Discussion A and the Cleveland, Harvard, and Pittsburgh groups acting concurrently to present Discussion B. It was also agreed that new material or rebuttal would appear in appendices.

It will be noted that, in addition to standard values for lipid measures derived from Donner data, certain other statistical data, the "estimated standard values" and the "estimated atherogenic indices," have been incorporated in Discussion A. Though based upon measurements made at the Harvard, Cleveland, and Pittsburgh laboratories on their referent populations, these estimated values and indices were computed independently by the Donner group. The Cleveland, Harvard, and Pittsburgh groups and the statistical consultant do not accept these "estimated standard values" and "estimated atherogenic indices" because they question the validity of the statistical methods by which they were derived.

Discussion A

Representing the views of John W. Gofman, Hardin B. Jones, Beverly Strisower, and Arthur R. Tamplin, *Donner Laboratory.*

The major purpose of this study was to explore further the early observations that an association existed between certain serum lipoproteins and existing coronary heart disease. The original observations indicated that a band of lipoproteins designated as S_f 10–20 lipoproteins was positively associated with this disease. At that time other flotation classes of lipoproteins were described, but measurements of their independent association with coronary heart disease were unavailable. It was planned originally to investigate the segments of the lipoprotein spectrum, other than the S_f 10–20 lipoproteins, and information is now available for all the major lipoprotein groups.

*Introduction of Standard Lipoprotein Measures**

Technical problems of measurement of lipoproteins with the ultracentrifuge were recognized and were in need of solution, since the method itself had been discovered approximately 1 year before this study was initiated. Among these problems were those of concentration measurement in the low concentration ranges and of the separation of lipoprotein classes. In the original work a preliminary ultracentrifugation was performed to concentrate the low-density lipoproteins 3-fold, in the effort to circumvent errors of measurement at low-lipoprotein concentration. The basic significant observations pertinent to coronary heart disease in association with S_f 10–20 lipoproteins were made under these conditions.[1] It became apparent that in some cases difficulty was being experienced in the measurement separation of the S_f 10–20 class of lipoproteins from the S_f 0–10 class. Therefore, the decision was made to measure S_f 12–20 lipoproteins instead of S_f 10–20 lipoproteins, in the effort to circumvent the difficulty of reading close to the prominent class of S_f 0–10 lipoproteins.

Further it was felt that possibly concentration of the lipoproteins 5-fold instead of 3-fold would further reduce measurement errors for low concentrations of lipoproteins. This innovation was made after the original work but before this cooperative study was initiated. It later became apparent that samples charac-

Technical Group as referee for the 2 discussions. He was assisted by 2 other members of the Committee on Lipoproteins and Atherosclerosis: Dr. John W. Fertig with regard to statistical matters and Dr. Jesse W. Beams with regard to questions involving physical theory of ultracentrifugation. Dr. Andrus was empowered to make final decision on admissibility of material in the discussions.

* Reproducibility for all measures in the Donner Laboratory is presented in table 22.

terized by high concentrations of low-density lipoproteins frequently gave false results for the S_f 12–20 lipoproteins when the 5-fold concentration was used instead of the 3-fold concentration, usually yielding falsely low S_f 12–20 results. Preliminary studies showed that a partial correction of this difficulty could be made by reanalyzing in the ultracentrifuge those samples characterized by high total lipoprotein concentration at a 3-fold concentration instead of 5-fold concentration. If such a scheme could be rigorously carried out, all samples would be analyzed at approximately equivalent total lipoprotein concentrations. However, a more definitive routine procedure was needed to handle this problem. In the course of 1951–1952 a highly refined ultracentrifugal analysis procedure was developed[4, 21] which took into account the fact that when lipoproteins are more concentrated in solution, they slow themselves down in flotation. This is akin to the well-known phenomenon of slowing of sedimentation of proteins in concentrated solution. Fortunately, the refined procedure resulted in no loss of previously made analyses, since the original film record (always on file) for an ultracentrifuge analysis could be reanalyzed by this refined procedure. Essentially this procedure corrects for the self-slowing of lipoproteins with increasing concentration of total lipoproteins and for a highly associated effect described as the Johnston-Ogston effect.[22] In order to denote that the refined procedure provides correct separation of the various flotation bands, no matter what the total lipoprotein concentration is in any sample, or whether 3-fold, 5-fold concentration, or other concentration is used, the designation *Standard flotation classes* was introduced. The need for the refined procedure was great, in that for samples of high total low-density lipoprotein concentration, a 100 per cent or even greater error of measurement was encountered.

With the refined analysis procedure, it became readily possible to evaluate other segments of the lipoprotein spectrum for association with coronary heart disease. It was demonstrated that the standard S_f 0–12, standard S_f 20–100, and standard S_f 100–400 lipoproteins showed positive and independent association with coronary disease as did the standard S_f 12–20 lipoproteins. Because of the large gain in information plus accuracy of analysis the Donner Laboratory proposed in 1952 that all the cooperating laboratories undertake the more refined measurement. Unfortunately the other laboratories felt unable to undertake the additional work necessary to provide the standard lipoprotein measurements, so that only the Donner Laboratory measurements are available for this study. However, as will be discussed, it is possible to make a partial *estimation* of the standard S_f 12–20 and standard S_f 20–100 results for the other laboratories by technics described below.

For the purposes of the Cooperative Study the introduction of the refined technic for standard lipoprotein measurements introduces no problem because of the careful precautions taken to insure that the study is a "blind study" with respect to the laboratories making the lipoprotein analyses. Thus all standard measurements as well as other measurements had to be registered in the office of the biometrician at the National Heart Institute in advance of the follow-up of cases for determination of development of new coronary heart disease.

Relationship of Lipoproteins and Cholesterol

Several studies in the literature, including those from Donner Laboratory, had indicated a positive association of analytic serum cholesterol measurement with coronary heart disease.[20, 23, 24] It was the intent of this study to compare and contrast the lipoprotein findings with those for serum cholesterol determination with respect to coronary disease.

Atherogenic Index Measurement

When it was discovered that all 4 standard lipoprotein classes, S_f 0–12, S_f 12–20, S_f 20–100, and S_f 100–400, showed positive and independent association with coronary heart disease, it became desirable to provide a combined measure for an individual subject, rather than to list separately each lipoprotein measure. While such a combined measure is desirable for estimating coronary disease risk, there is a wealth

of metabolic information pertinent to such problems as clinical management that resides in the individual lipoprotein class measurement.

The combined measure of lipoproteins predicting coronary disease risk has been designated the Atherogenic Index, or A.I., value. It is obtained directly from the standard S_f 0–12 and the combined standard S_f 12–400 lipoprotein measurements as follows.

Atherogenic Index or A.I. = 0.1 (standard S_f 0–12) + 0.175 (standard S_f 12–400)

where (standard S_f 0–12) = concentration of standard S_f 0–12 lipoproteins in mg/100 ml. and (standard S_f 12–400) = concentration of standard S_f 12–400 lipoproteins in mg/100 ml.

The weighting of the standard S_f 12–400 lipoproteins of 1.75 relative to that of the standard S_f 0–12 lipoproteins arises out of the fact that in applying Fisher's method,* such weighting resulted in the best segregation of a population sample of myocardial infarction and a matched control population sample without overt clinical coronary heart disease.

The A.I. measure represents the provision of information additional to that in a corrected S_f 12–20 determination, namely the standard S_f 12–20 measurement. Whereas the standard 12–20 measurement is broadly highly associated with coronary disease (and as this study indicates predicts coronary disease), we know that in certain individual cases the standard S_f 12–20 measurement alone cannot provide as good an evaluation of coronary disease risk as does the Atherogenic Index measurement.

The less refined S_f 12–20 measurement made at a 5-fold concentration presented in tables 14–17 is in our view a less adequate measure. It cannot be regarded as as critical an evaluation of the correct measure of S_f 12–20 lipoproteins, as the standard S_f 12–20 concentration. The standard S_f 20–100 level is correspondingly a better measure than the uncorrected S_f 20–100 measurement made at 5-fold concentration as recorded in tables 14–17.

Analysis of New Coronary Events

In the original work relating lipoproteins with coronary heart disease, every case was a documented myocardial infarction having both clinical and laboratory evidence to support the diagnosis.[1, 11] This was deemed essential to assure that the evaluation of possible association of lipoproteins with coronary disease was being made only with cases of the best order of diagnostic accuracy attainable. We would have preferred that this pattern of rigid documentation be followed in this critical test of the hypothesis for *de novo* coronary disease. In our opinion inclusion of cases of lesser diagnostic certainty such as those with "definite angina pectoris" (but no myocardial infarction) and "definite coronary disease by electrocardiographic evidence only" seriously dilutes the strongest clinical categories. The analysis of the new events by various clinical categories of certainty are presented in detail in Appendix A. We consider those data to provide the best appraisal of the follow-up studies of the relationship of lipoproteins and cholesterol with *de novo* coronary disease.

Since the other laboratories did not directly measure standard S_f 12–20, standard 20–100, or Atherogenic Index, we have given consideration to methods for obtaining the best estimate of such values from the data they did obtain. For estimation of standard S_f 12–20, the difference between measured standard 12–20 and uncorrected S_f 12–20 was evaluated as a function of serum cholesterol level. It would be anticipated that in general the difference between standard S_f 12–20 and uncorrected S_f 12–20 would increase with increasing cholesterol level, since standard S_f 0–12 levels correlate highly with cholesterol and since standard S_f 0–12 levels are responsible for errors in the S_f 12–20 measurement.

In this manner a set of equations† for cor-

* Fisher's method of Linear Discriminant Analysis was applied to develop the A. I. measurement.

† The following equations were used for all Donner sources, except for Los Angeles, and for all other laboratories:

S_f 12–20

For uncorrected 12–20 less than 70 mg. per cent:

(a) If cholesterol is less than 200 mg. per cent: *Estimated standard 12–20 = uncorrected 12–20 + 0.05 (cholesterol)*

(b) If cholesterol is greater than 200 mg. per cent: *estimated standard 12–20 = uncorrected 12–20 + 0.26 (cholesterol)-38*

For uncorrected 12–20 greater than 70 mg. per cent:

recting crude S_f 12–20 measures from the other laboratories to estimated standard S_f 12–20 and for correcting crude S_f 20–100 to estimated standard S_f 20–100 was obtained from the Framingham experience of the Donner base population. A separate set of equations was used to obtain estimated standard values for Los Angeles based upon the base population of that source. The application of the equations to the Donner sources other than Framingham indicated that the estimated standard 12–20 bore the same relationship to measured standard S_f 12–20 in these sources as it did in the source (Framingham) from which it was developed. Therefore there was excellent justification for the use of the equations to estimate standard S_f 12–20 and standard S_f 20–100 for the various clinical sources of all other laboratories.

It must be made clear at this point that while it is much better to have estimated standard 12–20 and estimated standard 20–100 values for the other laboratories in lieu of none, it could hardly be expected that the estimated standard values* would provide as much information with respect to such features as segregation of coronary events as do the measured standard S_f 12–20 and standard S_f 20–100 values.

In order to estimate the Atherogenic Index value for the laboratories where it was not directly measured, the cholesterol level was used as an estimate of standard S_f 0–12 lipoproteins, and the estimated standard 12–100 lipoproteins were used as a measure of the standard 12–400 lipoproteins. The sum of cholesterol plus estimated standard S_f 12–100 was the actual function used. This is analogous to a sigma function developed by Moore. On the Donner data it was demonstrated for each source that this composite measure was correlated with the measured Atherogenic Index to the extent of a Pearson correlation coefficient between 0.80 and 0.85. This composite measure was converted to a scale of A.I. units from the regression equation for A.I. on the composite measure as follows:

$$\textit{estimated A.I.} = 0.194\ (\textit{cholesterol} + \textit{estimated standard 12–100}) + 0.8$$

In this way it was possible to have an approximate Atherogenic Index for the other laboratories. While such an approximate value cannot be expected to provide as much information as a measured Atherogenic Index, it is of value in comparing expected Atherogenic Index results for all the laboratories.

The various lipid measures in the definite new events† are presented in tables 19 and 20

estimated standard 12–20 = uncorrected 12–20 + 0.20 (cholesterol)-47

S_f 20–100

For calculating estimated standard S_f 20–100.

(a) If uncorrected 20–100 is less than 70 mg. per cent: *estimated standard S_f 20–100 = uncorrected 20–100 + 0.16 (cholesterol)-31*

(b) If uncorrected 20–100 is between 70–159 mg. per cent:

For cholesterol below 230 mg. per cent: *estimated standard S_f 20–100 = uncorrected 20–100 − 2*

For cholesterol greater than 230 mg. per cent: *estimated standard S_f 20–100 = uncorrected 20–100 + 0.15 (cholesterol)-36*

(c) For uncorrected 20–100 greater than 160 mg. per cent: *estimated standard S_f 20–100 = uncorrected 20–100 − 0.15 (cholesterol)-31*

For the Los Angeles group (fasting bloods) the following equations apply:

S_f 12–20

For uncorrected 12–20 less than 60 mg. per cent: *estimated standard 12–20 = uncorrected 12–20 + 0.21 (cholesterol)-29*

For uncorrected 12–20 greater than 60 mg. per cent: *estimated standard 12–20 = uncorrected 12–20 + 0.13 (cholesterol)-22*

S_f 20–100

For uncorrected 20–100 less than 70 mg. per cent: *estimated standard 20–100 = uncorrected 20–100 + 0.16 (cholesterol)-18*

For uncorrected 20–100 between 70–159 mg. per cent: *estimated standard 20–100 = uncorrected 20–100 + 0.21 (cholesterol)-29*

For uncorrected 20–100 greater than 160 mg. per cent: *estimated standard 20–100 = uncorrected 20–100 − 0.15 (cholesterol)-31*

* Correlation coefficient:

estimated standard S_f 12–20 vs measured standard S_f 12–20 r = 0.73

estimated standard S_f 20–100 vs measured standard S_f 20–100 r = 0.73

(Correlation coefficients for Framingham, upon which the estimation equations were based are essentially identical with those for other sources (Eastman, Airlines) in which the equations were applied.)

† A complete roster of cases for which standard measurements and Atherogenic Index measurements are available is presented in table 18. In this discussion section the array used constitutes the first 3 categories combined of that table.

and the comparisons of various lipid measures with each other are in table 21.

The following conclusions can be drawn from these data:

1. The mean value for measured standard S_f 12–20 lipoproteins and for the measured Atherogenic Index is significantly elevated in the definite new events as compared with the base population for which such measurements were available.

2. The serum cholesterol for the *same group* of cases cannot be shown to be significantly elevated in the definite new events as compared with the base population.

3. Utilizing the estimated standard measurements and estimated Atherogenic Index values for all the laboratories the mean value for estimated standard S_f 12–20, the estimated Atherogenic Index, and the serum cholesterol level are each highly significantly elevated in comparison with the base population.

4. Segregation of definite new events above the fiftieth percentile is significant for standard S_f 12–20, standard S_f 20–100, standard S_f 12–100, Atherogenic Index, cholesterol, estimated standard S_f 12–20, estimated standard S_f 20–100, estimated standard S_f 12–100, and estimated Atherogenic Index.

5. Segregation of definite new events above the seventy-fifth percentile is significant (p = 0.03) only for the estimated Atherogenic Index. Both estimated standard S_f 12–20 and cholesterol are near the borderline of statistical significance (p = 0.06).

6. The standard S_f 12–20 measure is a highly significantly superior measure in contrast to the uncorrected S_f 12–20.

These conclusions may be summarized in

TABLE 18.—*Roster of Cases for which Standard Lipoprotein and Atherogenic Index Measures are Available*

	Standard S_f 12–20 mg./100 ml.	Standard S_f 20–100 mg./100 ml.	Atherogenic Index
Definite myocardial infarction or coronary thrombosis by clinical and laboratory evidence			
Donner, Los Angeles			
2-13898	65	139	97
2-16779	72	92	71
2-17494	130	136	104
2-30516	134	190	126
2-32104	72	110	98
2-07759	119	96	77
Donner, except Los Angeles			
2-17506	128	175	113
2-17517	76	110	77
2-17803	58	123	75
2-20307	81	166	98
2-20721	113	100	101
2-24423	67	128	81
2-25554	92	99	88
2-27664	110	125	110
2-34597	78	108	82
2-38879	65	134	97
2-42205	116	179	125
Angina pectoris			
Donner, Los Angeles			
2-27690	119	184	112
2-14685	47	90	91
2-18867	85	87	81
2-20866	88	91	74
2-27307	67	78	69
Donner, except Los Angeles			
2-24965	31	40	53
2-46031	63	125	79
Coronary disease by electrocardiographic evidence alone			
Donner, except Los Angeles			
2-15342	78	97	90
2-15444	56	99	81
2-29964	25	47	46
2-37479	49	85	60
New events retrospectively diagnosed as abnormal at entry			
Donner, Los Angeles			
2-16034	60	132	79
Donner, except Los Angeles			
2-09230	22	37	44
2-14954	69	168	110
2-15084	50	104	66
2-18672	45	148	105
2-22654	130	90	98
2-28521	99	123	109
2-39295	83	96	79
2-30259	85	112	97
2-33840	67	226	133
2-34969	157	181	120

Note: All tabulations and calculations in this table prepared at the Donner Laboratory.

TABLE 19.—*Mean Deviation in Lipid Level for Definite New Events from Levels for Base Populations: Various Lipid Measures, Men, 40–59*

(All Cases Having at Least the S_f 12–20, S_f 20–100 and Cholesterol Measures)

Measure and source	New events		Base population mean (mg./100 ml.)	Mean difference	
	Number	Mean (mg./100 ml.)		New events — base (mg./100 ml.)	p
Standard S_f 12–20 Donner	*28*			*10.7*	$p = 0.03$
Donner except Los Angeles	17	75.6	67.7	7.9	
Donner Los Angeles	11	90.7	75.7	15.0	
Standard S_f 20–100 Donner	*28*			*10.7*	x*
Donner except Los Angeles	17	114.1	106.2	7.9	
Donner Los Angeles	11	117.5	102.5	15.0	
Estimated standard S_f 12–20					
All Laboratories	57	76.7	66.9	9.8	$p < 0.01$
Estimated standard S_f 20–100					
All Laboratories	57	103.2	98.7	4.5	x
Atherogenic Index (A.I. Value)	*28*			*8.7*	$p = 0.04$
Donner except Los Angeles	17	85.6	79.6	6.0	
Donner Los Angeles	11	90.9	78.1	12.8	
Cholesterol (Donner Alone)	*28*			*7.2*	x
Donner except Los Angeles	17	251.0	241.3	9.7	
Donner Los Angeles	11	266.5	263.1	3.4	
Standard S_f 12–100 Donner	*28*			*21.5*	$p = 0.06$
Donner except Los Angeles	17	189.8	173.9	15.9	
Donner Los Angeles	11	208.3	178.2	30.1	
Estimated standard 12–100					
All Laboratories	57	180.1	165.6	14.5	$p = 0.06$
Estimated Atherogenic Index					
All Laboratories	57	87.9	80.3	7.6	$p < .01$

Note: All tabulations and calculations in this table prepared at the Donner Laboratory.
* p values greater than 0.10 are designated by x.

TABLE 20.—*Separation of Definite New Events at Specified Percentile Cutting Points: Various Lipid Measures, Men, 40–59*

Measure and source	Number of new events		
	Total	Above fiftieth percentile	Above seventy-fifth percentile
Donner			
Standard S_f 12–20	28	19 ($p = 0.05$)	9 x*
Standard S_f 20–100	28	19 ($p = 0.05$)	7 x
Standard S_f 12–100	28	22 ($p < 0.01$)	8 x
Atherogenic Index	28	21 ($p < 0.01$)	11 x
Cholesterol	28	19 ($p = 0.05$)	6 x
All laboratories			
Estimated standard S_f 12–20	57	39 ($p < 0.01$)	20 ($p = 0.06$)
Estimated standard S_f 20–100	57	36 ($p < 0.05$)	15 x
Estimated standard S_f 12–100	57	38 ($p = 0.01$)	17 x
Estimated Atherogenic Index	57	44 ($p < 0.01$)	21 ($p = 0.03$)
Cholesterol	57	41 ($p < 0.01$)	20 ($p = 0.06$)

Note: All tabulations and calculations in this table prepared at the Donner Laboratory.
* p values greater than 0.10 are designated by x.

TABLE 21.—*Comparisons of Specified Lipid Measures Based Upon Percentile Positions of Definite New Events, Men, 40–59*

Lipid measures compared		Number of events (N)	Mean percentile score		Mean difference A-B
Measure A	Measure B		Measure A	Measure B	
Donner					
Measured standard S_f 12–20	Cholesterol	28	61.9	56.5	+5.4
Measured standard S_f 20–100	Cholesterol	28	60.1	56.5	+3.6
Measured standard S_f 12–100	Cholesterol	28	61.9	56.5	+5.4
Measured Atherogenic Index	Cholesterol	28	63.3	56.5	+6.8
Standard 12–20	Uncorrected S_f 12–20	28	61.9	52.8	+9.1 $p < 0.01$
Standard S_f 20–100	Uncorrected S_f 20–100	28	60.9	56.2	+4.7
All sources					
Estimated standard 12–20	Cholesterol	57	59.5	61.3	−1.8
Estimated standard 20–100	Cholesterol	57	56.2	61.3	−5.1
Estimated standard 12–100	Cholesterol	57	58.8	61.3	−2.5
Estimated Atherogenic Index	Cholesterol	57	63.1	61.3	+1.8
Estimated standard 12–20	Uncorrected S_f 12–20	57	59.5	53.2	+6.3
Estimated standard 20–100	Uncorrected S_f 20–100	57	56.2	55.4	+0.8

Note: All tabulations and calculations in this table prepared at the Donner Laboratory.

TABLE 22.—*Summary of Within Laboratory (Donner) Reproducibility Studies, September 1951–June 1953*

Lipid measure	Period	Number of paired measurements	Technical error
S_f 12–20	9/51–6/53	264	5.6 mg./100 ml.
S_f 20–100	9/51–6/53	237	13.0 mg./100 ml.
Cholesterol	9/51–6/53	564	14.5 mg./100 ml.
Standard S_f 12–20*	9/51–6/53	135	7.4 mg./100 ml.
Standard S_f 20–100*	9/51–6/53	135	9.0 mg./100 ml.
Atherogenic Index*	9/51–6/53	135	5.2 A.I. units

Note: All tabulations and calculations in this table prepared at the Donner Laboratory.

* Samples used here are those involved in inter-laboratory reproducibility. However, since Donner alone did the standard measurements they provide data on intra-laboratory reproducibility.

the statement that there exists a predictive relationship of various blood lipid measures with *de novo* coronary disease. Indeed, it has been possible to demonstrate the relationship in spite of the dilution of the new events group by an appreciable number of cases of diagnostic certainty less than that for myocardial infarction or coronary thrombosis documented by clinical, laboratory, and electrocardiographic evidence. A much better measure of the true predictive relationship of blood lipid measures with *de novo* coronary disease and with each other is obtained by considering only the cases having definite clinical and laboratory evidence of myocardial infarction or coronary thrombosis. Such analysis is presented in Appendix A.

This Cooperative Study establishes clearly for the first time that elevation of blood lipids *precedes* clinical coronary disease and predicts it, rather than being a metabolic result of coronary disease.

DISCUSSION B

Representing the views of Lena A. Lewis, Frederick Olmsted, Irvine H. Page, *Cleveland Clinic*; Eleanor Y. Lawry, George V. Mann, Fredrick J. Stare, *Harvard School of*

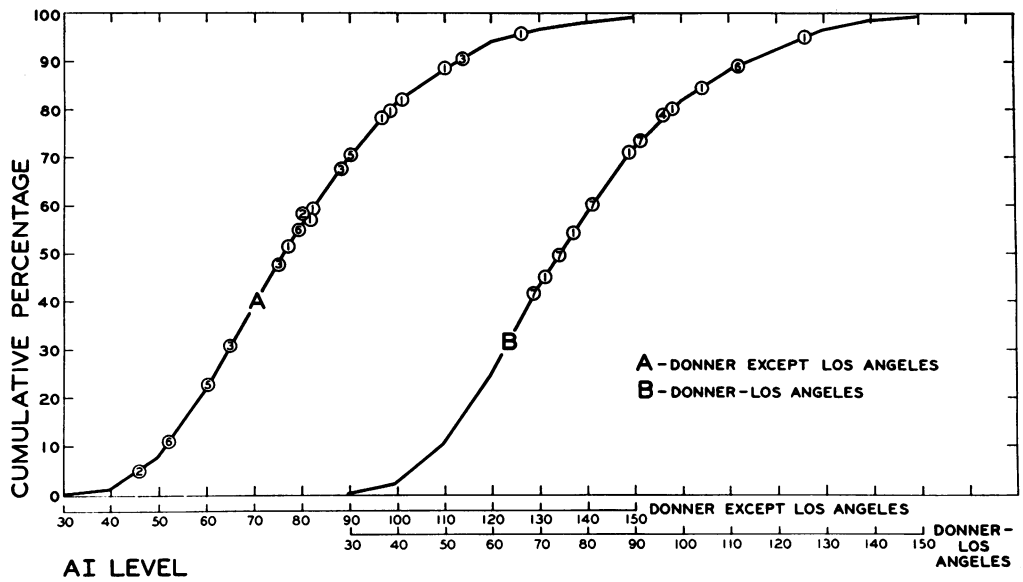

FIG. 7. Definite new events related to cumulative percentage of all subjects according to A. I. level, men 40–59.

Public Health; Martin Hanig, Max A. Lauffer, *University of Pittsburgh*; and Felix E. Moore, Statistical Consultant.

The data collected in this Cooperative Study indicate that the appearance of clinical manifestations of atherosclerosis is associated with prior elevation, on the average, of the total cholesterol and the S_f 20–100 lipoproteins. The possibility that the S_f 12–20 lipoproteins are also elevated cannot be ruled out, although this difference is small and statistical significance could not be shown. The present study does not confirm the hypothesis that lipid levels can successfully be used to predict those individuals who will develop coronary heart disease. The extent to which the various lipid measures failed in predictions is shown in figures 4–7.

This limitation of the lipid measures may also be illustrated by consideration of a hypothetical population of 1000 "well" men with an incidence of coronary heart disease, in relation to lipid values, similar to those studied here. Among 1000 "well" men of the ages studied there might be expected at most 20 new occurrences of coronary heart disease during 2 years of observation. By considering the half of the 1000 men with highest lipid levels as under suspicion from the outset, i.e., by setting the cutting point at the median, a correct prediction would have been made (depending on the lipid measure used) for only 10 to 14 of the 20 men who, in this hypothetical population, would have experienced a new event. Six to 10 of the new events, or a third to a half, would have been missed—these are the "false negatives." Almost 500 men, or about 50 per cent of the total population, would have been placed under suspicion but would not have experienced any event of clinical importance—these are the "false positives." The only way to diminish the number of "misses" or "false negatives" would be to set the cutting point at some position below the median, but this would further inflate the number of "false positives." It is this imprecision that casts serious doubt on the utility of these lipid measures in case finding or therapy.

The confirmation by the study that certain lipids are on the average elevated in advance of coronary heart disease may, however, be a useful finding for medical research, even though lipid measures do not permit clinically useful predictions for individuals. Even if the lipid

abnormality is not sufficiently distinctive, either in quality or quantity, to permit detection of individuals with imminent risk of attack by the disease, the differences in lipid levels that characterize groups of men may be useful in epidemiologic investigations of racial differences, sex differences, and perhaps of differences at more critical ages than were studied here. This use of lipid measurement may ultimately contribute substantially to the identification of causal factors in atherogenesis.

If there is a defect of lipid metabolism in coronary heart disease, why has this study demonstrated such a low association between elevated lipid levels and subsequent clinical events? There are several possible explanations.

The proper human population for an evaluation of measurements intended to discriminate between "well" persons and those afflicted with atherosclerosis—or prone to develop the clinical manifestations of atherosclerosis—may not be one composed of middle-aged, American men, for these are almost universally affected with the disease to some degree. Such extensive prevalence of the disease may prevent demonstration of a measurement-prediction relationship in the same way that an excessive dose may obscure evaluation of a response to a treatment. This limitation of the study population also applies to the premise that the lipid levels of the "well" or base populations are normal, and thus suitable referents for the subjects who developed coronary heart disease. The proper referent may lie outside that population, or in American subjects at a much earlier age, since there is important evidence that the anatomic changes of atherosclerosis begin early in life.[25, 26] Conceivably, also, atherogenesis is an episodic process with short-lived periods of advancement associated with high lipid levels and longer intervals of quiescence associated with low lipid levels. The limited available measurements of lipid levels of men at successive intervals do not, however, suggest such fluctuation. It may also be that the imprecision of methods, both laboratory and clinical, obscure the true relationship of lipid levels to coronary heart disease. The similarity of the several independent lipid measurements in classifying individuals seems to minimize this possibility.

It seems more reasonable, however, to assign at least a part of this lack of association of serum lipid levels with clinical events to the poorly understood phenomena that precipitate the occlusion of coronary flow. This event may or may not be thrombotic, and may bear little relationship to either the extent of atherosclerosis or serum lipid levels. It is well known that coronary insufficiency or occlusion may occur with either a minimim of strategically placed atherosclerosis, or it may fail to occur despite the most florid atheromatosis.

The original hypotheses of Gofman and his colleagues were based upon studies of groups of people with established evidences of coronary heart disease. The findings in these were compared with the measurements in "well" populations. Aside from the difficulty of avoiding the selection of those "after the fact" coronary subjects with gross derangements of serum lipid levels, there are other elements of selection that may prejudice the conclusions drawn. Such a diseased population must represent survivors. The existence of the diagnosis almost certainly assures that these people have been subjected to some kind of treatment and also establishes that the disease which they experienced must have been sufficient to have produced an important train of clinical events. All these factors may cause prospective and retrospective studies of coronary heart disease to be concerned with different kinds of people.

In their earliest report[1] the Donner group considered only disease subjects for whom there was "(a) a typical clinical history of myocardial infarction, (b) typical laboratory findings during the episode, and (c) electrocardiographic changes characteristic of myocardial infarction." These criteria were not defined nor were the data for individual subjects shown. But later in 1950[11] data were shown

that indicated that patients with "coronary insufficiency" also showed elevation of the S_f 10–20 class and this was taken as "further evidence consistent with the hypothesis that the molecules of S_f 10–20 class are associated with atherosclerosis." Again, in 1951[27] 63 patients with angina pectoris, who had not experienced an evident infarct were found to have *higher* levels of S_f 10–20 than did 203 men with established myocardial infarction. The "coronary cases" were combined and that practice was continued thereafter.[13] There is thus a clear precedent for the consideration of the kinds of definite coronary heart disease that the Review Committee accepted in the present work. A selection of some subgroup of these new events after the lipid data are correlated with clinical observations introduces the possibility of serious bias.

This arduous and expensive study has emphasized the limitations of the methods of lipid assessment that were used. These methods, and especially the lipoprotein methods, are exceedingly complex. Aside from the large capital outlay for equipment, the lipoprotein measurement requires a team of exceptionally well-trained personnel. The tasks are tedious and the "standard" measurements (S_f^0) require still another complex measurement and computation.

The origin of these "standard" measurements is of some interest. The Donner group asserts that their original investigation of the lipoproteins in human subjects, which led to the conclusion that certain serum lipoproteins were related to atherosclerosis, were done with serum aliquots of 3 ml. (C_o3). The original publication did not describe this important detail.[1] When the Cooperative Study was begun in 1950 the Donner Laboratory was using serum aliquots of 5 ml.[11] and this practice was recommended to the study. In 1951 and 1952, the Donner group observed that the data they were obtaining with 5-ml. serum aliquots did not confirm certain of their earlier observations. They concluded that this difference was was caused by the larger lipoprotein concentrations in the analytic cells that resulted from the larger serum aliquot. The Donner group then devised a method for compensating for these concentration effects and this process led to the "standard" measurements.

A general outline of this procedure was first presented to the Technical Group of the study in July 1952. Sufficient details for application of the method were made available by Donner in January 1953. The Donner group proposed that the study should adopt this new procedure and offered to carry out the measurements on the film records of the other laboratories. The majority did not agree that the methods of the Cooperative Study should be altered. Because of structural characteristics in the optical systems of half the centrifuges used in the eastern laboratories, these "standard" measurements could not in any event have been obtained from many of the film strips. The introduction of the calibration cell greatly facilitated the standardization of machine factors in the lipoprotein measurement. Nevertheless, the measurements in table 2 indicate that wide disparities then existed when aliquots of the same serum were analyzed in the various laboratories.

In a practical sense the lipoprotein measurements described in this work have serious disadvantages when compared with the measurement of cholesterol. The operation of the optical ultracentrifuge is costly and technically difficult. The cholesterol measurement, which is at least as useful, is better adpated to the facilities and production requirements of hospital laboratories.

The combinations of lipid measurements, either with one another or with observations of other kinds such as age or diastolic blood pressure, offer an almost endless variety of possible predictors. Each of these requires a new experiment for evaluation of its merits. Evaluation of 1 of these combined measures, the Atherogenic Index (A.I.), which was proposed by the Donner group in 1953,[12] has been

made on only the Donner data in the present study. Since computation of the A.I. requires the "standard" measurements and also the measurement of the S_f^0 0–12 and S_f^0 100–400 bands, the A.I. was not available from the Harvard, Pittsburgh, and Cleveland data. The Donner A.I. was not appreciably more successful in predicting definite new events than was the cholesterol measurement (table 21 in Discussion A).

This study was designed for the testing of certain rather specific hypotheses and for several reasons the data are not appropriate for making estimates of general incidence of coronary heart disease, even for the restricted age-sex group studied. The study population was of necessity drawn from groups where clinical follow-up would be available. The criteria for inclusion as a "well" person were intentionally set up to exclude many categories of borderline disease that might have caused difficulties of interpretation at follow-up, e.g., borderline high blood pressure. Finally, the evaluation of new events was very rigorous in order to exclude any event from the "definite" category about which there might be a reasonable doubt.

In summary, the authors of this discussion conclude:

Atherosclerosis, as manifested by definite evidence of coronary artery disease, was associated with an antecedent elevation of the serum S_f 20–100 (and possibly S_f 12–20) lipoproteins and of the serum cholesterol.

The elevation of serum lipoprotein and cholesterol was not of clinical use in predicting those individuals who would develop coronary heart disease during the observation period.

The demonstration of elevations of cholesterol and lipoproteins in the serum of groups of men who developed coronary heart disease may have a useful application in epidemiologic studies of heart disease.

The difficulty of demonstrating an association of serum lipids with atherogenesis may be related to the inappropriate use of an American population, which is almost universally affected with atherosclerosis.

The use of S_f 12–20 and S_f 20–100 lipoprotein measures, or the related Atherogenic Index (A.I.), had no advantage over the simpler measurement of cholesterol in the characterization of men prone to develop coronary heart disease.

The lipoprotein measurements are so complex that it cannot be reasonably expected that they could be done reliably in hospital laboratories.

REFERENCES

[1] GOFMAN, J., LINDGREN, F., ELLIOTT, H. MANTZ, W., HEWITT, J., STRISOWER, B., AND HERRING, V.: The role of lipids and lipoproteins in atherosclerosis. Science **111:** 166, 1950.

[2] DAWBER, T. R., MEADORS, G. F., AND MOORE, F. E.: Epidemiological approaches to heart disease: The Framingham study. Am. J. Pub. Health **41:** 279, 1951.

[3] PHILLIPS, E., CHAPMAN, J. M., AND GOERKE, L. S.: Relative values of technique used in detection of heart disease. Am. Heart J. **45:** 319, 1953.

[4] DELALLA, O., AND GOFMAN, J.: Ultracentrifugal analysis of human serum lipoprotein. *In* Methods of Biochemical Analysis, vol. 1, ed. by D. Glick. New York, Interscience, 1954.

[5] MACINNES, D. A.: Introduction to the conference on the ultracentrifuge. Ann. N. Y. Acad. Sc. **43:** 175, 1942.

[6] HANIG, M., AND SHAINOFF, J. R.: Specific refractive increment of serum lipoproteins obtained by flotation. J. Biol. Chem. **219:** 479, 1956.

[7] ARMSTRONG, S. H., BUDKA, M. J. E., MORRISON, K. C., AND HASSON, M.: Preparation and properties of serum and plasma proteins. XII. The refractive properties of the proteins of human plasma and certain purified fractions. J. Am. Chem. Soc. **69:** 1747, 1947.

[8] Minutes of the meeting of the Technical Group, Committee on Lipoproteins and Atherosclerosis, Berkeley, Calif., July 26–27, 1951.

[9] Minutes of the meeting of the Committee on Lipoproteins and Atherosclerosis. Cleveland, Ohio, November 8, 1951.

[10] Minutes of the meeting of the Technical Group, Committee on Lipoproteins and Atherosclerosis, Chicago, Ill., June 11, 1952.

[11] GOFMAN, J., JONES, H., LINDGREN, F., LYON, T. ELLIOTT, H., and STRISOWER, B.: Blood lipids and human atherosclerosis. Circulation **2:** 161, 1950.

[12] —, STRISOWER, B., DE LALLA, O., TAMPLIN, A., JONES, H., AND LINDGREN, F.: Index of coronary artery atherogenesis. Mod. Med. **11:** 119, 1953.

[13] —, GLAZIER, F., TAMPLIN, A., STRISOWER, B., DE LALLA, O.: Lipoproteins, coronary heart disease, and atherosclerosis. Physiol. Rev. **34:** 589, 1954.

[14] ABELL, L., LEVY, B., BRODIE, B., AND KENDALL, F.: A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. J. Biol. Chem. **195:** 357, 1952.

[15] COLEMAN, D. M., AND MCPHEE, A. F.: An improved method for determination of total serum cholesterol. Am. J. Clin. Pathol. **26:** 181, 1956.

[16a] SCHOENHEIMER, R., AND SPERRY, W. M.: A new method for determination of free and combined cholesterol. J. Biol. Chem. **106:** 745, 1934.

[16b] SPERRY, W. M., AND BRAND, F. C.: The colorimetric determination of cholesterol. J. Biol. Chem. **150:** 315, 1943.

[17] KEITH, N. M., WAGENER, H. P., AND BARKER, N. W.: Some different types of essential hypertension: their course and prognosis. Am. J. M. Sc. **197:** 332, 1939.

[18] GLAZIER, F., TAMPLIN, A., STRISOWER, B., DE LALLA, O., GOFMAN, J., DAWBER, T,, AND PHILLIPS, E.: Human serum lipoprotein concentrations. J. Gerontol. **9:** 395, 1954.

[19] KEYS, A., MICKELSEN, O., MILLER, E., HAYES, E., AND TODD, R.: The concentration of cholesterol in the blood serum of normal men and its relation to age. J. Clin. Invest. **29:** 1347, 1950.

[20] JONES, H., GOFMAN, J., LINDGREN, F., LYON, T., GRAHAM, D., STRISOWER, B., AND NICHOLS, A. V.: Lipoproteins in atherosclerosis. Am. J. Med. **11:** 358, 1951.

[21] GOFMAN, J., JONES, H., LYON, T., LINDGREN, F., STRISOWER, B., COLMAN, D., AND HERRING, V.: Blood lipids and human atherosclerosis. Circulation **5:** 119, 1952.

[22] JOHNSTON, J. P., AND OGSTON, A. G.: Boundary anomaly found in ultracentrifugal sedimentation of mixtures. Tr. Faraday Soc. **42:** 789, 1946.

[23] MORRISON, L., HALL, L., AND CHENEY, A.: Cholesterol metabolism. Am. J. M. Sc. **216:** 32, 1948.

[24] GERTLER, M., GARN, S., AND LERMAN, J.: Interrelationships of serum cholesterol, cholesterol esters and phospholipids in health and in coronary artery disease. Circulation **2:** 205, 1950.

[25] ENOS, W., HOLMES, R., AND BEYER, J.: Coronary disease among United States soldiers killed in action in Korea. J. A. M. A. **152:** 1090, 1953.

[26] STRONG, J., MCGILL, H., GRIFFIN, O., AND HOLMAN, R.: Natural history of aortic atherosclerosis, ages 1 to 40. Abstracted, Fed. Proc. **15:** 533, 1956.

[27] GOFMAN, J., LINDGREN, F., JONES, H., LYON, T., AND STRISOWER, B.: Lipoproteins and atherosclerosis. J. Gerontol. **6:** 105, 1951.

APPENDIX A*

John W. Gofman, Hardin B. Jones, Beverly Strisower and Arthur R. Tamplin, *Donner Laboratory.*

The findings presented in the basic report demonstrate a predictive relationship of blood lipid measures with *de novo* coronary disease. Of importance is an evaluation of the strength of this predictive relationship and of the relative merits of various blood lipid measures in such prediction. Further it is of great clinical importance to know the separate relationships between blood lipids and such entities as myocardial infarction or coronary thrombosis established by clinical and laboratory evidence, angina pectoris, and simple electrocardiographic evidence of coronary disease.

The lipid measures in the various categories of diagnosis are presented in table A-1. Inspection of the ranking of cases shows clearly that definite myocardial infarction or coronary thrombosis by clinical and laboratory evidence is characterized by higher blood lipid levels, for any lipid measure used, than either angina pectoris alone or electrocardiographic evidence alone of coronary disease. Neither angina pectoris alone nor electrocardiographic evidence alone nor the combination of both categories can be proven within these data to have lipid values higher than the base population. In contrast, there is striking segregation of the definite myocardial infarction cases based upon clinical plus laboratory evidence from the base population. Our interpretation of those findings is that a clinical diagnosis based upon the subjective finding of angina pectoris alone or of electrocardiographic evidence alone cannot be accepted as adequate evidence of that process, associated with blood lipids, presumably atherosclerosis, which leads to myocardial infarction or coronary thrombosis. It should be noted here again that the original hypothesis of an

* All tabulations and calculations on Appendix A prepared at the Donner Laboratory.

TABLE A-1.—*Comparison of Various Diagnostic Categories with Respect to Blood Lipid Measurements, All Laboratories*

Category A vs. Category B	Percentile Ranking				Significance of Difference in Segregation
	Category A		Category B		
	Above fiftieth	Below fiftieth	Above fiftieth	Below fiftieth	
Lipid Measure					
Cholesterol	32	5	4	7	$p < 0.01$
Estimated Atherogenic Index	33	4	6	5	$p = 0.03$
Estimated standard 12–20	31	6	4	7	$p < 0.01$
Estimated standard S_f 20–100	28	9	6	5	x*
Estimated standard S_f 12–100	30	7	5	6	$p = 0.03$
Category A vs. Category C	**Percentile Ranking**				
	Category A		Category C		
	Above fiftieth	Below fiftieth	Above fiftieth	Below fiftieth	
Lipid Measure					
Cholesterol	32	5	5	4	$p = 0.08$
Estimated Atherogenic Index	33	4	5	4	$p = 0.06$
Estimated standard 12–20	31	6	4	5	$p = 0.04$
Estimated standard 20–100	28	9	2	7	$p < 0.01$
Estimated standard 12–100	30	7	3	6	$p < 0.02$

Category A = Definite myocardial infarction or coronary thrombosis by clinical plus laboratory evidence.

Category B = Angina pectoris.

Category C = Electrocardiographic evidence as the only evidence of coronary disease.

* p values greater than 0.10 are designated by x.

association of blood lipids with coronary heart disease was developed using only myocardial infarction cases documented by clinical, laboratory, and electrocardiographic evidence. At no time in all the investigations of the Donner group has electrocardiographic evidence alone been considered as an adequate criterion for inclusion in a series of cases of coronary heart disease. Lyon (A-1)* recently showed that those angina pectoris cases that did develop myocardial infarction subsequently were characterized by strikingly higher Atherogenic Index values than those angina pectoris cases remaining uncomplicated.

Additional Evidence for the Association of Blood Lipids with Coronary Disease

In table A-2 are presented the data for a very interesting series of cases. All these cases were originally and specifically classified by their respective referring sources as free of cardiovascular disease and hence were eligible for the normal pool of 40–59 year old men. In the follow-up the referring sources indicated that there was now evidence of clinical coronary disease. Hence these represented cases for consideration for inclusion among the *de novo* events. However, upon the basis of information obtained by the referring sources, by the statistical consultant, or by the Review Committee a decision was made by that Committee, though without foreknowledge of their respective lipid patterns, that these cases should have been considered abnormal at entry to the study. Such retrospective diagnosis may be exceedingly dangerous because of the possibility that some bias may be brought into the selection of cases. Since every effort was made at the original examination to exclude "abnormals," this should clearly have been the time for a final decision with respect to eligibility for inclusion or exclusion of the case with respect to the normal pool. Analysis of the lipid findings in this group of "retrospective abnormals" is presented in table A-2. It is found that in this independent group of cases the Atherogenic Index is highly significantly elevated, whereas the cholesterol level is not significantly different from that for the base population. In fact this group of cases, retrospectively removed from the study, is characterized by as high Atherogenic Index values as any group in this over-all study. The removal of these cases retrospectively has the effect of minimizing the observed lipoprotein elevation in *de novo*

* Reference notes preceded by A- refer to Appendix references only. Other numbered references will be found in bibliography of basic report.

TABLE A-2.—*Mean Deviation in Lipid Level for "Retrospective Abnormals" from Levels for Base Populations, Various Lipid Measures, Men, 40–59 years*

Measure	New events		Base population Mean	Mean difference New events — base	
	Number	Mean			
S_f 12–20, all sources	24	43.0	44.0	−1.0	x*
S_f 20–100, all sources	24	114.2	99.9	+14.3	x
Cholesterol, all sources	23	251.0	240.9	+10.1	x
Cholesterol, (Donner—cases for which A.I. and Cholesterol are both available)	11	249.5	243.3	+ 6.2	x
Atherogenic Index					
Donner, all cases	12	95.3	79.4	+15.9	$p = 0.02$
Donner, cases for which cholesterol is available	11	94.5	79.3	+15.2	$0.03 > p > 0.02$

* p values greater than 0.10 are designated by x.

events and maximizing the serum cholesterol elevation. Since the number of cases so removed is an appreciable fraction of the total, the seriousness of possible bias cannot be overestimated. Whatever is done with these "retrospective abnormals" in this follow-up study, it is quite clear that lipoprotein-Atherogenic Index measurement showed an ability to segregate them. Cholesterol level failed to accomplish this.

"Strongest" de novo Cases

As referred to several times above, the cases of definite myocardial infarction or coronary thrombosis by clinical plus laboratory evidence represents the group of greatest diagnostic certainty and also the group that corresponds to that upon which the hypothesis of association of blood lipids with coronary disease was developed. This group of cases should logically represent the central core of cases upon which the follow-up evaluation should be based. Furthermore, independent logic can be used, based upon the findings that the strongest cases are characterized by different lipid values from those for cases that the Review Committee itself classified as of lesser diagnostic certainty. Fortunately, the number of cases in the "strongest event" category is adequate to test several of the critical issues for which this whole study was designed. That these issues could not be resolved as well in the array of cases presented in the foregoing sections can be directly ascribed to the dilution effect of inclusion of relatively large numbers of cases of less certain diagnosis.

Analysis of the Strongest New Events

In consideration of the segregation of new events from the base population it is, of course, of importance to compare the results in such new events with the hypothesis that was advanced. Thus, since the hypothesis that lipoprotein levels segregate "coronaries" from "noncoronaries" clearly stated that some cases of definite myocardial infarction show low lipoprotein levels it would not be expected that, in this test of *de novo* events, *all* the cases should show marked elevation of the lipid measure under consideration. The question becomes one of determining whether a predictive measurement will do as well in a new population as it has done in study populations of established coronary disease. These questions can be answered by statistical analysis of the new events to determine: 1. Is the mean level of lipids in the *de novo* events that which had been found for the study population of myocardial infarcts? 2. How do the various lipid measures compare in segregation power? 3. Is the distribution of lipid values in the *de novo* population that which had been found for the study population? 4. Is the prediction of coronary risk as a function of lipid value (A.I. for example) in the *de novo* events in agreement with that which was found in the study population?

The extent of segregation of the new events by measurement level can be subjected to statistical test if some arbitrary percentile is chosen for a comparison of the "success" of a measure in associating a high level with the subsequent development of cardiovascular disease. The fiftieth and seventy-fifth percentiles have been chosen for the present comparison. When the observed and expected number of definite new events are compared by χ^2 tests, table A-3, the following results are obtained:

At the seventy-fifth percentile cutting points, the Atherogenic Index (Donner), the estimated Atherogenic Index (all laboratories) and serum cholesterol showed segregation of the strongest events from the base population at or below the $p = 0.01$ level. Standard S_f 12–20 and standard S_f 20–100 showed significant segregation also ($p = 0.04$).

At the fiftieth percentile cutting points, the Atherogenic Index, the estimated Atherogenic Index, the standard S_f 12–20, the standard S_f 20–100,

TABLE A-3.—*Significance Tests Based Upon Separation of Strongest New Events at the 50th and 75th Percentile Cutting Points**

Lipid measures	Number of new events			p	Number of new events			p
	Total	Above seventy-fifth percentile	Below seventy-fifth percentile		Total	Above fiftieth percentile	Below fiftieth percentile	
Atherogenic Index (A.I. Value) (Donner)	17	10	7	<0.01	17	15	2	<0.01
Estimated Atherogenic Index (All Sources ‡)	37	17	20	<0.01	37	33	4	<0.01
Standard S_f 12–20 (Donner)	17	8	9	0.04	17	15	2	<0.01
Standard S_f 20–100 (Donner)	17	8	9	0.04	17	15	2	<0.01
S_f 12–20 (Uncorrected for flotation-concentration dependence, all sources)‡	37	14	23	0.01 > p > 0.05	37	21	16	x†
S_f 20–100 (Uncorrected for flotation-concentration dependence, all sources)‡	37	11	26	x†	37	28	9	<0.01
Cholesterol (All sources)‡	37	16	21	0.01	37	32	5	<0.01

* Based upon one-sided χ^2 test.

† x Indicates $p > 0.10$.

‡ All sources = Pooled data from Cleveland, Harvard, Donner, and Pittsburgh laboratories.

All percentile values are referred to appropriate base population.

the S_f 20–100 and serum cholesterol all showed segregation of strong new events from the base population at or below the $p = 0.01$ level. For the various lipid measures the paucity of cases falling below the fiftieth percentile is noteworthy and indicative of the predictive strength of the blood lipid measures. For the entire 37 cases from all laboratories pooled, only 1 case fell below the twenty-fifth percentile on the estimated Atherogenic Index measurement, and only 2 cases fell below the twenty-fifth percentile on the estimated Atherogenic Index measurement and only 2 cases fell below the twenty-fifth percentile on the cholesterol measurement. The relative freedom of persons in the lowest 25 per cent of the distribution of such lipid measures from development of serious coronary disease is striking.

Comparison of the Various Lipid Measures in Segregation Power

One objective of the cooperative study was to compare the ultracentrifugal lipoprotein measurement with the serum cholesterol measure with respect to segregation power. Two ultracentrifugal measures are pertinent in this regard, the standard S_f 12–20 and the Atherogenic Index (A.I.) value. The comparison of these measures with serum cholesterol for the strongest events are presented in table A-4. The data of this table indicate that the Atherogenic Index and standard S_f 12–20 measurements are significantly superior to the serum cholesterol measure in segregation of the strongest new events. In the previous sections of this report, additional evidence in this same direction was presented, although with the small number of cases available, significance tests in each individual instance were not below the $p = 0.05$ level.

There are fundamental considerations based upon studies of the chemical structure of lipoproteins that bear directly on the question of the information potentially obtainable from a measurement of serum lipoproteins as compared with a measurement of serum cholesterol. These considerations indicate that it would be impossible for the serum cholesterol neasure to be more strongly related to coronary heart disease than the serum lipoprotein measurement. On the other hand, these same fundamental considerations show readily why, in accord with the results presented here and in previously published data, the serum lipoprotein measure would be expected to show a stronger relationship with coronary disease than the cholesterol measure.

Essentially, no one today questions the fact that cholesterol in the serum is present as a constituent of several lipoprotein classes. There is no cholesterol present in nonlipoprotein form. Logically then one may deduce that if cholesterol is of any importance with respect to coronary disease, which it is, then such importance must arise because at least certain of the serum lipoproteins are related to coronary disease. For the high-density lipoproteins (which

TABLE A-4.—*Comparison of Specified Lipid Measures, Men, 40–59, Based Upon Percentile Position of Strongest New Events*

Lipid measures compared		Number of events	Mean percentile score		Mean difference A-B	p
Measure A	Measure B		Measure A	Measure B		
Atherogenic Index	Cholesterol	17	72.8	62.8	+10.0	0.05
Standard S_f 12–20	Cholesterol	17	73.6	62.8	+10.8	0.05

Comparison of Specified Lipid Measures Based Upon Placement of Strongest New Events Relative to Seventy-fifth Percentile

Measure A	Measure B	Agreement. Both measures on same side of cutting point			Disagreement. One measure below, the other above cutting point			p
		Total	Both above	Both below	Total	A above	B above	
Atherogenic Index	Cholesterol	8	3	5	8	7	1	$0.1 > p > 0.05$
Standard S_f 12–20	Cholesterol	9	2	7	8	6	2	x*

* p values greater than 0.10 are designated by x.

correspond to α-lipoproteins by other technics) what evidence there exists points to a *lower level* of such lipoproteins in coronary disease than in matched controls. This means that the cholesterol measure will either be unaffected or will actually be lowered in coronary disease with respect to that portion of the cholesterol in high-density (α) lipoproteins. But a far larger and more important effect is to be considered with respect to the low-density lipoproteins. The standard S_f 0–12 lipoproteins contain 34 per cent cholesterol by weight and the standard S_f 12–400 lipoproteins contain 13 per cent cholesterol by weight. Therefore 100 mg. per cent of standard S_f 0–12 lipoproteins contain an amount of cholesterol equivalent to 260 mg. per cent of standard S_f 12–400 lipoproteins. Published evidence[12] indicates that the standard S_f 12–400 lipoproteins are approximately 1.75 times as important, milligram for milligram, as standard S_f 0–12 lipoproteins. The confidence limits on this relative importance factor make it extremely unlikely that the factor could be as low as 1.0. Even if it were as low as 1.0, it is evident that the most unfavorable situation with respect to lipoproteins would still result in the lipoprotein measure being more highly related to coronary disease than the cholesterol measure. This is so because in any coronary series studied, some cases show predominant standard S_f 0–12 elevation, others, standard S_f 12–400 elevation, and others an elevation in both classes. With *any* elevation in standard S_f 12–400 in coronary disease, the low relative abundance of cholesterol in these lipoproteins makes it imperative that the lipoprotein over-all measure must be superior to the cholesterol measure. The converse situation, of a superiority of cholesterol over lipoproteins is an impossibility. Should a particular population sample be selected where an apparent superiority of cholesterol measure over the lipoprotein measure is found, the only possible explanation is some type of technical bias in the study. The remote possibility that the standard S_f 12–400 lipoproteins are less important, milligram for milligram, than the standard S_f 0–12 lipoproteins still leaves the situation unaltered. For under such circumstances the cholesterol measure would be underestimating the standard S_f 0–12 lipoproteins and hence would still be an inferior measure in comparison with a measure of the lipoprotein spectrum. Indeed the closer the standard S_f 12–400 lipoproteins approached 0 importance or conversely the closer the standard S_f 0–12 lipoproteins approached 0 importance, the more inferior the cholesterol measure would be to a lipoprotein measurement of both standard S_f 0–12 and standard S_f 12–400 classes.

It is not surprising in this study to find that the lipoprotein Atherogenic Index measure shows superior segregation to the cholesterol measure, since the logical considerations above indicate it could not have resulted otherwise. Indeed, it was by no means necessary to depend upon this study for the purpose of comparing the cholesterol and the lipoprotein measurements. The crucial issue of this cooperative study was to establish whether the blood lipid elevation, either of cholesterol or of lipoproteins, *precedes* clinical coronary disease, rather than follows it. Once this is established, it follows from elementary logic that lipoprotein measurement, adequately performed, must result in superior segregation of coronary disease over the

cholesterol measurement. Any consideration of a possible superiority of the cholesterol measure is equivalent to the acceptance of a physical impossibility.

Comparison of the de novo Population Results with the Myocardial Infarction Population in the Original Hypothesis

1. Is the mean level of lipids in the *de novo* strongest events that which had been found for the study population of myocardial infarcts?

A.I. Value (40–59 year Men)

	n	*Mean A.I. units*
Published data for myocardial infarctions[12]	239	92.5
De novo strongest events in this study	17	95.3
Base population (same for both studies)		79.5

The difference between the *de novo* definite events and the base population is 15.8 units, which is significant beyond the $p = 0.01$ level. The *de novo* definite events show a mean level 2.8 A.I. units higher than the study population of myocardial infarctions, but this cannot be proven significant. Thus, the findings with respect to A.I. value in the *de novo* events is completely consistent with the hypothesis that had been advanced.

Estimated A.I. value (all Laboratories)

	n	*Mean estimated A.I. units*
For strongest cases	37	100.3
Base population		80.3
Difference		20 units

These findings for estimated A.I. values for all laboratories are consistent with the A.I. values measured for the Donner Laboratory and with the data for the postinfarction study group.

The hypothesis that myocardial infarction is characterized by elevation in standard S_f 12–20 lipoproteins is substantiated by the *de novo* strongest events. The improvement effected by correction of the S_f 12–20 for flotation-concentration dependence is evident in comparison of the *de novo* events for standard S_f 12–20 and S_f 12–20 (see table 21).

Cholesterol levels. Post myocardial infarction patients show elevated serum cholesterol levels in comparison with matched base populations.[20, 23, 24] The present study shows that the serum cholesterol in the strongest new events is elevated in comparison with the base population. The differences between myocardial infarctions and their base populations are as follows:

Males 40–59 Years

	n	Cholesterol Levels Difference in means *Coronary-Noncoronary*
Published data (Donner Laboratory[20]) for already established myocardial infarction 40–59 yr males	156	27.3 mg./100 ml.
Definite new events in this study 40–59 yr. men, all sources	37	32.7 mg./100 ml.

It is evident that the findings in the *de novo* events are completely consistent with the hypothesis that elevated serum cholesterol is found in persons developing myocardial infarction.

2. Is the distribution of lipid values in the *de novo* population that which had been found for the study population of myocardial infarction?

The standard deviations in the distribution of lipid measures in the new events and in study populations of myocardial infarction are presented below;

A.I. Value (40–59 year Men)

Donner published standard deviation of distribution (A-2) on 239 myocardial infarction cases	= 20 units
Standard deviation in 17 *de novo* definite events (this study) (Donner)	= 20 units
S_f 12–20 (40–59 year men)	
Donner published standard deviation in 156 myocardial infarctions[20]	34 mg./100 ml.
Standard deviation 37 *de novo* events in this study (all sources)	30 mg./100 ml.
Serum Cholesterol Levels	
Donner published standard deviations in 156 myocardial infarctions[20]	62 mg./100 ml.
Standard deviation in 37 *de novo* events in this study (all sources)	49 mg./100 ml.

It is apparent that the standard deviation of the distribution of lipid measures in the definite new events reported in this study are comparable with those for earlier study populations of postmyocardial infarction patients.

3. Is the prediction of coronary risk as a function of lipid value in the *de novo* events in agreement with that found in the study population?

Since the mean values and the standard deviation of the distributions of lipid measures in the *de novo* events are within expectable agreement with those for study populations, prediction of coronary risk as a function of lipid level would be expected to be similar from the *de novo* data as from the study population data. Specifically, the Donner Labora-

tory has published coronary risk data as a function of the A.I. value.[12]

The *de novo* definite events may be used to test this published predicted risk. For this purpose the twenty-fifth, fiftieth, and seventy-fifth percentile will be considered. From the published graph of risk of coronary disease as a function of A.I. value, one may calculate what the expected distribution of *de novo* definite coronary events would be and compare this with observation.

A.I. Values	Observed in the 17 de novo cases	Expected from published Data
Above twenty-fifth percentile	17	16
Below twenty-fifth percentile	0	1
Above fiftieth percentile	15	14
Below fiftieth percentile	2	3
Above seventy-fifth percentile	10	9
Below seventy-fifth percentile	7	8

It is seen that the risk of coronary disease rises as a function of A.I. value, and that the observed relative risks in the *de novo* events are consistent at the twenty-fifth, fiftieth, and seventy-fifth percentile with previously published risks based upon postmyocardial infarction study cases.

SUMMARY

Blood lipid measures are distinctly shown by this study to be predictive of coronary heart disease, rather than the result of coronary heart disease.

The predictive power of serum lipoprotein and and Atherogenic Index measurement, and serum cholesterol measurement are consistent with estimates published on the basis of study of postmyocardial infarction patients. The lipoprotein-Atherogenic Index measure is superior to the cholesterol measure in predictive power.

The predictive power of such measurements is high and hence should be of great clinical usefulness in preselection of individuals with high risk of future overt coronary heart disease.

Blood lipid measurements are higher in the well-documented cases of myocardial infarction and coronary thrombosis than in either angina pectoris or individuals considered to show coronary disease based upon electrocardiographic evidence alone.

Apparently well individuals characterized by high lipoprotein Atherogenic Index or cholesterol measurements should not be considered "normal," but rather to have an elevated risk of future coronary disease.

REFERENCES APPENDIX A

A-1 LYON, T. P., YANKLEY, A., GOFMAN, J. W., AND STRISOWER, B.: Lipoproteins and diet in coronary heart disease. A five-year study. California Med. **84:** 325, 1956.

A-2 GOFMAN, J. W., TAMPLIN, A., AND STRISOWER, B.: Relation of fat and calorie intake to atherosclerosis. J. Am. Dietetic Assoc. **30:** 317, 1954.

APPENDIX B

Lena A. Lewis, Frederick Olmsted, Irvine H. Page, *Cleveland Clinic*; Eleanor Y. Lawry, George V. Mann, Frederick J. Stare, *Harvard School of Public Health*; Martin Hanig, Max A. Lauffer, *University of Pittsburgh*; Felix E. Moore, Statistical Consultant; and J. Franklin Yeager, Administrative Officer

It is stated in Discussion A that "In the course of 1951–1952 a highly refined ultracentrifugal analysis procedure was developed[4, 21] which took into account the fact that when lipoproteins are more concentrated in solution, they slow themselves down in flotation." The reader might get the impression that the "standard" lipoprotein methods were described in the 1952 report.[21]* Actually these methods were first described in general terms to the Technical Group on June 11, 1952[10]; the details of the method were received by the statistical consultant on January 14, 1953 and were published in 1954.[4] Almost all of the lipid data included in the present report had been collected before mid-1953. The representatives of the Eastern laboratories held that introduction of the corrections at that time as proposed by Donner would have been equivalent to a change in the hypothesis.

The Donner group now proposes that the appraisal of the predictive value of lipoproteins be based solely on the results for the 37 men in the Cooperative Study who developed "definite myocardial infarction," "definite coronary thrombosis," or "definite coronary sclerosis," thus excluding data for 20 new events assigned by the Review Committee to the following classifications: "myocardial infarction, definite by ECG only," "ECG abnormality, definite, associated with coronary artery disease," "angina pectoris, definite, with ECG changes," or "angina pectoris, definite, without ECG changes."† This limiting of the type of case is not in accord either with general understandings or the specific agreements of the Technical Group of the Committee on Lipoproteins and Atherosclerosis.

Between August 4, 1955 and April 11, 1956, the Review Committee had examined the records of

* References refer to the bibliography of the main report.

† The Donner group has also excluded eight new events for which no cholesterol measurements are available.

EXHIBITS

FORM NO 1 (Rev. 5-52)

COOPERATIVE STUDY OF LIPOPROTEINS AND ATHEROSCLEROSIS
INITIAL CASE REPORT FORM

1. SOURCE		2. DATE	FOR CODING USE ONLY
3. NAME: FIRST LAST		4. NUMBER	
5. SEX ☐ MALE ☐ FEMALE	6. RACE ☐ WHITE ☐ OTHER	7. AGE	
8. DATE LAST EXAMINATION	9. HEIGHT	10. WEIGHT	
11. HAS THIS PERSON A PREVIOUS HISTORY, OR ARE THERE PHYSICAL OR LABORATORY FINDINGS SUGGESTING CARDIOVASCULAR DISEASE, DIABETES OR NEPHRITIS? ☐ NO ☐ YES	IF YES, SPECIFY:		
12. ECG FINDINGS: ☐ NORMAL ☐ ABNORMAL ☐ QUESTIONABLE ☐ NOT DONE			
13. URINALYSIS: a. ALBUMIN	b. SUGAR	14. BLOOD PRESSURE	
15. OTHER SIGNIFICANT FINDINGS:			
LABORATORY DATA			
16A. Sf 20-100			
16B. Sf 12-20	17. TOT. CHOLESTEROL	18. OTHER	

FORM NO. 2.

CLINICAL REAPPRAISAL
COOPERATIVE STUDY OF LIPOPROTEINS AND ATHEROSCLEROSIS

1. NAME 2.

3. DATE OF CLINICAL REAPPRAISAL OF THIS SUBJECT

4. HAS THIS PERSON HAD ANY EVIDENCE OF MYOCARDIAL INFARCTION, ANGINA PECTORIS, OR CEREBRO-VASCULAR ACCIDENT SINCE DATE OF INITIAL REPORT ?

☐ NO ☐ YES ☐ QUESTIONABLE ☐ DATA UNOBTAINABLE

IF ANSWER IS YES OR QUESTIONABLE, COMPLETE FORM NO. 3 WHICH ITEMIZES THE DETAILS

5. IF UNABLE TO OBTAIN INFORMATION, STATE REASON, (IF NON-CARDIOVASCULAR DEATH, STATE DATE AND CAUSE.)

6. OTHER INFORMATION

EXHIBIT A.—*Facsimiles of Forms 1 and 2*

For a subject to be included in the study, the following items had to be completed: Form No. 1: In addition to the serial number, assigned by the laboratory, items 1, 2, 3 (or 4), 5, 7, 11, 16B. Form No. 2: Items 1 (or 2), 3, 4.

all new events and had classified them according to the categories listed in table 10; the Committee's classification and the lipid values had been reported to the laboratories by January 26, 1956 for all except 2 of the 65 definite new events ultimately included in the study. The proposal to limit the analysis to the 37 cases described above was made by Donner in a letter dated April 14, 1956. The Eastern laboratories held that the proposal for shifting a classification scheme of such fundamental importance to the Study should have been made before practically all the data were in. At this point in the course of the Study, the Eastern laboratories did not find this change in classification acceptable because they believed it unsound to change the method of analysis after the results were examined.

The Donner group cites the *Science* paper of 1950[1] and the *Circulation* paper of 1950[11] as proof that definite infarction, thrombosis, and sclerosis were the only kinds of disease used originally and thus the only kinds admissible now. Perusal of reference 11 reveals that patients with uncomplicated angina pectoris showed lipoprotein levels not different from those of patients with myocardial infarction; in subsequent publications both patients with myocardial infarction and those with angina pectoris were used in discussion and in the derivation of the Atherogenic Index.[13, 21, 27]

In Appendix A the contention is made that the results of this Study may have been biased—in a statistical sense—by the exclusion of persons originally classified as normal (possibly by clerical error) on Form 1 who were later reclassified by the Review Committee as "abnormal at entry." The precautions that were taken to avoid bias have been described in the section "Evaluation of bias" in the body of the report. The Review Committee, consisting of Dr. Paul D. White, Chairman, Dr. Samuel A. Levine, and Dr. Howard B. Sprague, which was charged with the responsibility for passing on eligibility of reported new events for inclusion in the Study as well as classification of the type of clinical event, did not have access to the lipoprotein or cholesterol values. Furthermore, consideration of the 24 subjects disqualified as abnormal at entry by the Review Committee (see Exhibit F) indicates that only 9 would have been classified in the "definite events" categories listed in table 10. Inclusion of these 9 cases in the analysis would not have altered our conclusions.

Finally, we believe the data show clearly that neither the measurement of lipoprotein nor that of cholesterol provided predictive value for individuals during the 2-year observation period of this study.

EXHIBIT B.—*Electrocardiographic Abnormalities Disqualifying for the Normal Pool*

1. Ventricular tachycardia
2. Ventricular fibrillation
3. Incomplete A-V block (prolonged A-V or P-R conduction time)
4. Incomplete A-V block with dropped beats
5. Complete A-V block
6. Bundle-branch block, left
7. Bundle-branch block, right
8. Intraventricular block, unclassified
9. Intraventricular block, intermittent
10. Elevation of S-T junction (J)
11. Depression of S-T junction (J)
12. Inverted T wave except in lead III, aV_R, V_1 or V_2.

All other electrocardiographic abnormalities will be rated in accordance with the '2 out of 3' rule; e.g., disqualifying if BP greater than 145/95.

EXHIBIT C.—*Mean Lipid Values According to Follow-up of Men, 40–59 in mg./100 ml. Serum*

	Number of cases				Mean values				Standard deviation followed and pooled
	All cases	Followed and pooled	Followed not pooled*	Not followed	All cases	Followed and pooled	Followed not pooled*	Not followed	
S_f 12–20—all Sources	6133	4914	761	458	43.2	44.0	40.1	40.6	23.4†
Cleveland	1280	974	195	111	33.9	34.3	33.1	31.9	22.7
Donner	2530	2088	233	209	50.0	50.8	46.5	45.8	24.5
except Los Angeles	1817	1402	233	182	47.9	48.4	46.5	45.7	23.2
Los Angeles	713	686	—	27	55.4	55.8	—	46.7	26.2
Harvard	1157	843	242	72	39.3	39.4	39.0	40.2	20.4
Pittsburgh	1166	1009	91	66	42.6	42.9	42.0	39.4	23.8
except prisoners	840	773	35	32	45.0	45.0	46.5	43.8	24.8
Prisoners	326	236	56	34	36.5	36.1	39.1	35.3	18.8
S_f 20–100—all Sources	6119	4903	759	457	94.2	94.6	93.0	91.9	69.0†
Cleveland	1272	968	194	110	85.2	84.4	89.6	84.6	63.3
Donner	2530	2088	233	209	99.9	100.3	103.1	91.6	72.6
except Los Angeles	1817	1402	233	182	106.2	108.1	103.1	94.9	76.7
Los Angeles	713	686	—	27	83.8	84.4	—	69.1	60.4
Harvard	1156	842	242	72	85.7	85.0	85.1	95.6	60.1
Pittsburgh	1161	1005	90	66	100.2	100.6	95.5	100.9	73.1
except prisoners	836	769	35	32	101.7	101.9	95.3	102.7	76.7
Prisoners	325	236	55	34	96.5	96.3	95.6	99.3	59.8
Cholesterol—all Sources	4909	3985	539	385	237.2	237.4	235.2	238.2	48.0†
Cleveland	684	475	111	98	241.6	241.4	243.9	239.8	50.0
Donner	2016	1704	160	152	247.4	248.2	237.8	248.5	51.5
except Los Angeles	1450	1163	160	127	241.2	241.3	237.8	244.3	46.8
Los Angeles	566	541	—	25	263.4	263.1	—	269.4	57.7
Harvard	1046	799	178	69	242.4	242.7	239.8	245.3	46.2
Pittsburgh	1163	1007	90	66	212.3	212.9	210.5	204.6	42.4
except prisoners	837	771	34	32	218.5	218.5	226.9	209.8	42.6
Prisoners	326	236	56	34	196.1	194.6	200.6	199.8	36.3

* Not pooled because of irregular follow-up intervals.

† Calculated on the assumption of a common variance but different laboratory means.

EXHIBIT D.—*New Events as Classified by the Review Committee, Men, 40–59*

Case #	Source	Age	Interval (yrs.:mos.)	Absolute values			Percentiles			Event*
				S_f 12–20	S_f 20–100	chol.	S_f 12–20	S_f 20–100	chol.	
Definite Events										
1-01013	Cleve. Graph. Bronze	54	1:06	31	110	—	53	73	—	1
1-01398	Cleve. Graph. Bronze	44	1:09	34	158	—	59	90	—	1
1-01523	Cleve. Graph. Bronze	46	1:11	61	146	252	90	86	60	1
1-01616	Cleve. Graph. Bronze	44	0:07	91	326	—	98	99	—	1
1-02012	GE-Schenectady	50	2:01	57	17	325	88	9	95	1
1-02472	GE-Nela Park	50	0:07	43	185	—	75−	93	—	1
1-02515	GE-Nela Park	56	1:11	22	91	—	31	64	—	1
1-02628	GE-Nela Park	40	2:02	68	176	342	93	92	97	1
1-04284	Nickel Plate	59	0:02	17	71	161	20	51	2	7
1-04285	Nickel Plate	58	1:00	13	99	166	13	68	4	3
1-04287	Nickel Plate	54	0:04	57	170	264	88	92	69	1
1-04783	Cleve. Graph. Bronze	47	0:07	10	77	—	6	54	—	1
2-07081	Pan American	53	0:07	58	56	—	75−	21	—	3
2-07759	Los Angeles	56	1:03	47	68	286	44	50+	68	1
2-09510	Los Angeles	50	0:02	59	151	—	63	90	—	1
2-13898	Los Angeles	51	0:05	59	104	306	63	75+	79	4
2-14685	Los Angeles	56	1:00	34	73	342	18	54	92	7
2-15342	Eastman Kodak	55	3:06	47	80	271	57	41	77	5
2-15444	Framingham	52	<2:01	34	79	264	24	40	72	2
2-16779	Los Angeles	49	1:00	50	99	235	49	72	33	1
2-17494	Los Angeles	57	0:07	93	51	281	92	29	65	1
2-17506	Eastman Kodak	46	3:05	82	77	262	93	38	71	3
2-17517	Eastman Kodak	42	2:08	43	109	256	48	63	65	1
2-17803	Pan American	49	0:06	37	131	220	34	74	33	3
2-18867	Los Angeles	54	0:11	37	56	185	24	35	7	7
2-20307	Eastman Kodak	49	3:02	75	164	284	90	86	83	1
2-20721	Eastman Kodak	45	0:05	45	131	244	53	74	56	1
2-20866	Los Angeles	58	1:02	68	75	250	74	56	43	7
2-24423	Framingham	51	0:11	49	107	266	61	61	73	1
2-24965	Eastman Kodak	56	2:05	29	29	198	16	3	17	6
2-25554	Framingham	44	0:10	41	75	274	44	36	79	3
2-27307	Los Angeles	59	0:11	39	67	280	28	49	64	7
2-27664	Framingham	51	1:04	62	118	257	79	68	66	1
2-27690	Los Angeles	54	1:01	114	90	274	96	66	59	6
2-29964	Framingham	56	2:01	19	43	193	3	10	14	2
2-30516	Los Angeles	58	0:02	67	144	285	73	89	67	1
2-32104	Los Angeles	40	0:04	45	133	207	41	86	15	1
2-34597	Framingham	57	1:02	52	99	253	68	55	63	1
2-37479	Framingham	56	1:00	36	73	209-F	30	35	24	5
2-38879	Framingham	47	0:09	43	172	247	48	87	59	1
2-42205	Pan American	51	1:08	83	131	335	93	74	97	1
2-46031	Framingham	59	1:02	37	120	234	34	69	46	6
3-04071	MIT	56	2:03	51	67	260	78	46	68	7
3-08302	MIT	40	0:05	44	99	363	68	73	99	1
3-08367	Rexall	46	1:09	30	44	218	38	21	29	5
3-08777	Met. Life	48	<1:01	24	29	206	22	9	19	5
3-09002	Met. Life	48	2:00	29	70	290	35	49	86	1
3-09135	Oxford	46	1:00	59	275	250	86	99	60	1
3-11493	Met. Life	47	0:07	62	123	292	88	83	86	1
3-12129	Met. Life	44	0:06	17	75	260	7	54	68	1

EXHIBIT D—*Continued*

Case #	Source	Age	Interval (yrs.:mos.)	Absolute values			Percentiles			Event*
				S_f 12–20	S_f 20–100	chol.	S_f 12–20	S_f 20–100	chol.	
Definite Events										
4-00911	Armco	45	1:09	38	138	276	44	80	91	2
4-02118	Atlanta†	53	0:08	230	339	278	100	98	92	1
4-02285	Atlanta‡	48	<2:00	21	64	157	11	33	5	7
4-02365	Atlanta†	46	1:08	58	132	196	78	78	31	1
4-02666	Leavenworth‡	42	1:05	33	91	225	34	55	57	1
4-03002	Hoffman	55	1:11	33	16	319	34	2	99	1
4-03103	Weirton	53	1:01	20	63	251	9	32	80	1
4-03316	Weirton	48	0:07	41	113	254	49	70	81	1
4-03935	Weirton	58	0:11	106	92	239	98	55	73	1
4-03944	Weirton	44	2:00	14	42	207	3	15	41	6
4-04386	Ford	47	1:07	53	62	408	73	31	100	1
4-04430	Hoffman	53	1:11	40	71	241	48	38	74	5
4-04480	Weirton	45	2:01	77	168	255	91	87	82	5
4-04578	Weirton	48	1:07	34	90	256	36	54	82	1
4-04977	Weirton	43	1:04	34	54	247	36	25	78	3
Probable or Possible Events										
1-02593	GE-Nela Park	50	1:04	46	27	335	78	15	97	9
1-03913	Chrsyler	51	2:02	80	172	272	96	92	75+	9
1-04695	Cleve. Graph. Bronze	41	2:00	29	13	—	48	7	—	9
2-13055	United Airlines	45	<1:06	71	223	—	88	94	—	14
2-13538	Eastman Kodak	55	<2:02	47	109	227	57	63	40	10
2-17475	Los Angeles	59	1:03	53	34	179	55	12	5	12
2-21346	Framingham	57	1:03	45	80	194	53	41	15	15
2-22442	Eastman Kodak	48	3:00	52	108	239	68	62	51	15
2-26981	Los Angeles	57	0:09	28	40	232	10	16	30	11
3-07323	Rexall	42	0:04	30	75	241	38	54	52	11
3-08546	Met. Life	48	2:03	71	134	241	92	86	52	13
3-08986	Met. Life	41	<2:00	33	79	196	45	57	15	11
3-12658	Met. Life	45	0:09	73	89	363	93	65	99	15
4-02277	Atlanta‡	58	<2:00	33	59	198	34	28	33	15
4-02961	Leavenworth‡	45	1:10	37	74	198	42	41	33	11
4-03480	Weirton	59	1:03	17	49	163	6	20	7	8
4-05308	Weirton	48	0:08	82	251	302	94	96	97	9

(F)—Framingham cholesterol used in absence of Donner.
* Code for Committee Classification given in table 10.
† Civil service.
‡ Prisoner.

EXHIBIT E.—*New Events Reported for White Motors*

Case #	Age	Interval (yrs.:mos.)	S_f 12–20	S_f 20–100	Reported event
1-00188	49	1:00	43	211	Myocardial infarction
1-00198	58	1:06	35	13	Def. myocardial infarction*
1-00498	43	1:04	26	−0	Myocardial infarction
1-00573	28	0:11	<12	74	Quest. coronary occl.
1-00702	51	1:04	35	29	Def. myocardial infarction*
1-00762	58	—	42	84	Coronary thrombosis
1-00840	56	1:02	26	13	Acute coronary insuff.

* So classified by Review Committee.
Note: Cholesterol values are not available for cases from White Motors.

EXHIBIT F.—*Cases Considered Abnormal at Entry by the Review Committee, Men, 40–59*

Case #	Source	Age	S_f 12–20	S_f 20–100	Chol.	Evidence indicating person abnormal at entry	Classification of event‡
1-02058	GE-Schenectady	49	32	95	228	Questionable ECG at admission	Angina pectoris, definite, with ECG changes
1-02241	GE-Schenectady	44	29	42	231	Possibly abnormal ECG at entry	ECG abnormality, probable, assoc. with coronary insufficiency
1-04291	Nickel Plate	52	13	32	268	Abnormal ECG at entry	Myocardial infarction, definite
2-09230	Framingham	54	19	36	242	Pre-existing AP	Angina pectoris, definite, w/o ECG changes
2-14954	Eastman Kodak	58	57	227	192	Persistent hypertension	CVA, definite
2-15084	Pan American	55	52	85	234	Pre-existing AP	Insufficient evidence of disease
2-16034	Los Angeles	58	52	164	227	Pre-existing AP	Angina pectoris, definite, w/o ECG changes
2-18672	Eastman Kodak	46	43	254	198	Possible Buerger's disease at entry	Peripheral vascular disease
2-22654	Framingham	46	39	80	319	Pre-existing coronary insufficiency	Angina pectoris, probable, w/o ECG changes
2-28521	Framingham	54	39	133	284	Intermittent claudication at entry	Coronary thrombosis, probable
2-30259	Framingham	46	37	146	276	Pre-existing ? AP	Angina pectoris, possible
2-31017	Los Angeles	55	56	92	—	Pre-existing AP	Coronary thrombosis, definite
2-33840	Framingham	48	82	363	242-F	Pre-existing ? AP	Angina pectoris, definite, w/o ECG changes
2-34969	Framingham	57	73	122	304	Pre-existing ? AP	Angina pectoris, definite, w/o ECG changes
2-39295	Framingham	54	41	77	226-F	Abnormal ECG at entry	Coronary thrombosis, probable
3-09787	Oxford	45	30	63	254	Abnormal ECG at entry	Coronary thrombosis, definite
4-02111	Atlanta*	55	45	97	215	Pre-existing AP	Myocardial infarction, definite
4-02127	Atlanta*	45	29	43	201	Pre-existing AP	Angina pectoris, probable, w/o ECG changes
4-02964	Leavenworth†	41	25	70	187	Pre-existing AP	Angina pectoris, probable, with ECG changes
4-03469	Weirton	51	40	134	255	Questionable ECG at admission	Myocardial infarction, probable by ECG only
4-03809	Weirton	43	26	48	190	Abnormal ECG at entry	ECG abnormality, probable, assoc. with coronary artery disease§
4-03957	Weirton	53	57	64	299	Abnormal ECG at entry	ECG abnormality, possible, assoc. with coronary artery disease
4-04304	Weirton	48	42	54	278	Abnormal ECG at entry	ECG abnormality, probable, assoc. with coronary artery disease§
4-05103	Weirton	57	75	219	235	Entry ECG suspicious	ECG abnormality, probable, assoc. with coronary artery disease

* Civil Service.

† Prisoner.

‡ Dr. Paul D. White, Chairman of the Review Committee, assigned the classifications which in his opinion would have been given by the Committee if the cases had not been rejected because of abnormality at entry.

§ Right bundle-branch block.

(F)—Framingham cholesterol used in absence of Donner.

EXHIBIT G.—*"Standard" Lipid Measures: Supplement to Exhibit D, Donner Only: Men, 40–59*

Case #	Source	Absolute values			Percentiles		
		"Standard" S_f 12–20	"Standard" S_f 20–100	AI	"Standard" S_f 12–20	"Standard" S_f 20–100	AI
Definite Events							
2-07081	Pan American	74	85	65	63	36	31
2-07759	Los Angeles	119	96	77	91	54	54
2-09510	Los Angeles	60	143	89	36	81	71
2-13898	Los Angeles	65	139	97	42	79	79
2-14685	Los Angeles	47	90	91	18	50−	73
2-15342	Eastman Kodak	78	97	90	69	49	70
2-15444	Framingham	56	99	81	37	51	58
2-16779	Los Angeles	72	92	71	51	51	45
2-17494	Los Angeles	130	136	104	94	77	85
2-17506	Eastman Kodak	128	175	113	97	91	91
2-17517	Eastman Kodak	76	110	77	66	61	51
2-17803	Pan American	58	123	75	41	71	48
2-18867	Los Angeles	85	87	81	68	48	60
2-20307	Eastman Kodak	81	166	98	71	90	80
2-20721	Eastman Kodak	113	100	101	92	52	82
2-20866	Los Angeles	88	91	74	71	50+	49
2-24423	Framingham	67	128	81	54	74	58
2-24965	Eastman Kodak	31	40	53	6	3	11
2-25554	Framingham	92	99	88	81	51	68
2-27307	Los Angeles	67	78	69	45	42	42
2-27664	Framingham	110	125	110	91	73	88
2-27690	Los Angeles	119	184	112	91	90	89
2-29964	Framingham	25	47	46	2	6	5
2-30516	Los Angeles	134	190	126	95	92	95
2-32104	Los Angeles	72	110	98	51	63	80
2-34597	Framingham	78	108	82	69	59	59
2-37479	Framingham	49	85	60	27	36	22
2-38879	Framingham	65	134	97	51	78	79
2-42205	Pan American	116	179	125	93	92	96
2-46031	Framingham	63	125	79	47	73	54
Probable or Possible Events							
2-13055	United Airlines	72	170	94	60	91	75+
2-13538	Eastman Kodak	63	105	70	47	56	40
2-17475	Los Angeles	53	68	54	26	31	17
2-21346	Framingham	54	99	61	33	51	24
2-22442	Eastman Kodak	96	99	90	84	51	70
2-26981	Los Angeles	52	63	68	24	25	40

ACKNOWLEDGMENT

In a study of this magnitude, which extends over several years, many people make substantial contributions. It is not possible to name all of those who have contributed, but particular mention should be made of 2 groups: 1. Our associated laboratory, clinical, and statistical personnel. 2. The directors of medical departments in the organizations that have provided the serum samples and the medical information used in the study.

ASSOCIATED PERSONNEL

Cleveland

STEPHAN BARANY
HELEN BROWN
LAVERNE FISHER
HEDDY RABE

Harvard

MARTHA BARBER
DOROTHY DUNBAR
ROBERT KENNEY
DONALD McCLEAN
RITA O'CONNELL
ANN PETERSON
ALICE WYSOCKI

Donner

ALICE CHIN
DAVID COLEMAN
ARDRA CORNELIUS
OLIVER deLALLA
FRANK GLAZIER
VIRGIL HERRING
FRANK LINDGREN
ALICE McPHEE
ALEX NICHOLS
GEORGE OKAWACHI
BETTY PIMENTAL
FRED STAUFFER
BEVERLY STRISOWER
ARTHUR TAMPLIN
ROBERT TANDY

Pittsburgh

MARCEL A. BALUDA
CARL B. CARLSON
MARYELLEN CARROLL
S. WALTER ENGLANDER
FREDERICK R. FRANKE
CHARLES C. HOHING
ROBERT E. KILBURN
S. WAYNE KLEIN
D. F. KOENIG
ALEXANDER D. LOWY, JR.
CAMPBELL MOSES, JR.
A. W. PETRE
JOHN R. SHAINOFF
EDMUND TAYLOR
MARTHA WHEATLEY

Biometrics Research Section, National Heart Institute

TAVIA GORDON
MARY ANN HALPERIN
IRENE LIMA
DORSEY OFFUTT
PHILIP PERSON

MEDICAL DIRECTORS AT THE SOURCES

Cleveland

White Motor Co.—*G. F. Sykes*†
Chrysler Motor Company—*M. W. Jocz*
Cleveland Graphite Bronze—*J. C. Placak*
General Electric Co.—Schenectady—*W. F. MacDonald*, Nela Park—*E. Kline*
Nickel Plate Railroad—*John Houk*

Donner

Framingham Heart Study—*Thomas Dawber*
City of Los Angeles—*Edward Phillips*
Pan American Airways—*Frederick Leeds*
United Air Lines—*A. C. Ladd*
Eastman Kodak Co.—*David W. Fassett*

Harvard

American Mutual Ins. Co.—*R. C. Williamson*
Dr. Burwell—*C. Sidney Burwell*
Campbell Soup Co.—*J. M. Kimmich*
Dr. Chapman—*E. M. Chapman*
Lahey Clinic—*F. N. Allan*
Metropolitan Life Ins. Co.—*K. J. Thomson*
Mass. Inst. of Technology—*Dana Farnsworth*
Rexall Drug Co.—*Fenn Poole*†
Standard Oil of N. J.—*Robert Page*
Swift and Co.—*H. V. Somers*
Oxford Diabetes Study—*Hugh Wilkerson*

Pittsburgh

Hoffmann-LaRoche, Inc.—*E. D. Kyhos*
Weirton Steel Co.—*J. L. Thompson*
Federal Prisons—Atlanta—*H. M. Janney*, Leavenworth—*R. O. Settle*, Terre Haute—*T. H. Smith*
Westinghouse Electric Co.—*F. R. Gray*
Armco Steel Corp.—*J. A. Llewellyn*
Ford Engineering Co.—*J. H. Weidner*

† Deceased.

LIST OF TABLES

LIST OF EXHIBITS

LIST OF FIGURES