Serum Cholesterol and Relative Body Weight of Coronary Patients in Different Populations

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Comparisons of coronary patients with their clinically healthy counterparts in areas where coronary heart disease is extremely common show that the patients tend to have serum total cholesterol concentrations higher than the "controls." Prospective studies in the United States show that this tendency exists before the disease is clinically apparent.

Similarly, populations that apparently differ in the frequency of coronary heart disease show corresponding differences in the average serum cholesterol values of men of given age in those populations.

These facts and other evidence lead to the hypothesis that the serum cholesterol concentration is an important causal factor in the development of atherosclerosis and its complications in the form of clinical coronary heart disease. Not unnaturally, this view is sometimes interpreted to mean that the disease is a result of a "metabolic fault" that produces cholesterol levels above some critical level at which atherosclerosis is promoted. In turn, the objection is raised that many coronary patients are reported to have cholesterol levels that are not particularly high in comparison with the general population or presumed "normal" for the U.S. But there is very little information on coronary patients in populations in which the disease is less common than here.

While most investigators now accept the view that serum cholesterol does play a role in the etiology of coronary heart disease, there seems to be even wider acceptance of the view that overweight is contributory. The prospective studies mentioned above indicate that overweight has much the same predictive value for future clinical disease as does the cholesterol level; perhaps more influential is the evidence from the insurance industry that the risk of future mortality among insured policy holders is increased with increasing levels of relative body weight at the time of the insurance examination. We have pointed out some of the weaknesses of the conclusions from these insurance data but the massive numbers of policy holders involved are impressive and undoubtedly justify actuarial conclusions about the risk of mortality of insurance applicants.

 Naturally, therefore, there is great interest in obtaining data on "normal" values of serum cholesterol and relative body weights and on these characteristics in coronary patients, particularly since both of these characteristics are readily modified by the diet. These efforts so far have been largely confined to the analysis of data from the United States and a few similar "high coronary" populations. But it is instructive to inquire into the characteristics of men with coronary heart disease, compared with suitable controls, in other populations as well as in the United States. The present paper reports findings in Naples, Italy, and in the Twin Cities of Minneapolis and St. Paul, Minnesota.

**Subjects and Methods**

**Coronary Patients**

Any study in the United States on serum lipids and overweight in coronary patients is currently apt to be seriously complicated by the increasing tendency of physicians to prescribe, and the patients themselves to adopt, dietary or pharmaceutical programs to reduce the blood cholesterol level or the body weight or both. During the past
few years more than half of the coronary patients from the Twin Cities seen in the Laboratory of Physiological Hygiene have made some dietary change before we saw them. Accordingly, for the present analysis we have elected to concentrate on coronary patients studied in the years 1953 to 1955, at which time our methods were standardized in the present form but deliberate attempts to control blood cholesterol and body weight in coronary patients were much less common than now. A secondary series of patients studied in 1955 to 1957 will be noted also.

In Naples, Italy, relatively little emphasis has been given until very recently to cholesterol and weight control of coronary patients, and dietary alterations after the appearance of the clinical disease did not appreciably affect the series in the years 1957 to 1959, which are reported here.

The upper age limit of 65 years was set for the coronary patients and all but 4 of the total of 218 patients thus included were 40 years or more, 2 of these men being in the Twin Cities and 2 in Naples.

The Twin Cities coronary patients were urban residents whose occupation was of the white-collar type—business, professional, and clerical—and none had been engaged in manual labor. No special selection was made, however, in regard to social or economic status. They were seen through the cooperation of several local internists who were asked to refer their male coronary patients up to age 65 who could be brought to the Laboratory of Physiological Hygiene for examination. They were asked not to refer patients because of known abnormality of lipid metabolism and to exclude patients in whom the coexistence of disease besides coronary heart disease might be a complicating factor. Further, they were requested not to refer patients for whom they had already prescribed dietetic or medication programs designed to control blood cholesterol or body weight.

These stipulations were adhered to in general, but several patients had to be excluded when we discovered exceptions. Several other patients were excluded because they were not clearly cases of coronary heart disease.

The medical criterion for inclusion in the Twin Cities coronary series was clear evidence of previous myocardial infarction or of classical Heberden's angina pectoris. Almost all the patients in the latter category exhibited one or another electrocardiographic abnormality, depressed S-T segments and T waves if not QRS indications of old infarction.

The Neapolitan coronary patients were seen through the cooperation of Dr. Mario Mattioli and the internists in charge of cardiovascular patients in the University Clinics. Only patients with clear evidence of previous myocardial infarction were included. Because myocardial infarction in the general poor population of Naples is apparently much less common than among the wealthy class there or than in Minnesota, special efforts were made to seek out patients in the working classes. For this and other reasons, the social class distribution of coronary patients studied in Naples may not be representative of the true distribution of infarction by socioeconomic class in that city.

Control Subjects

In order to match the age range of the coronary patients the men to be considered as control subjects were restricted to ages 40 to 66. They were all fully employed at the time of study and were judged to be clinically healthy on the basis of medical examinations.

The Twin Cities control subjects were men engaged in professional, business, or clerical work in the Twin Cities, none was engaged in manual labor, and they were considered to correspond reasonably closely to the coronary patients in social and economic status. Both control and coronary series included some business executives, physicians, and wealthy men and, in general, were probably more representative of the upper class of the Twin Cities than of the general population.

The Twin Cities control subjects were almost all secured by invitation of men whose names were on rosters of employees supplied by 28 corporations in Minneapolis and St. Paul. About 90 per cent of the invitations were accepted. In addition to those on these rosters, about 10 per cent of the Twin Cities control subjects were self-employed or employees of firms other than the major list of corporations who had learned of our program from friends on the roster and were included at their request after we were convinced they were not simply hypochondriac or otherwise peculiar.

In Naples it was considered essential to secure control subjects in several socioeconomic classes. There is a major difference between the upper class of business and professional men and the general population of the working class whose mode of life is greatly different in several respects, notably in the diet. Further, within the general working class distinction should be made between clerical workers and other relatively sedentary men of the poor class on the one hand and men doing heavy manual work on the other, even though in Naples there is little difference in economic status between these types.

Accordingly, business and professional men were recruited through the Rotary Club of Naples and through professional colleagues. Several cases of coronary heart disease were found in this group (and were included in the coronary series), and the remainder are considered as "controls." Cleri-
eral workers were secured through cooperation of the City and State Tax Offices of Naples. Most of the men accepted the opportunity of examination, without deduction of pay for time off the job.

Physically active workers in Naples were represented by 3 groups of men. Workers of one shift at one section of the Ilva steel mills were examined on the premises and, because of convenience and no loss of pay for time off, few declined. The second group consisted of regular members of the City Fire Brigade stationed at their main station and headquarters a few yards from the Institute of Physiology. Over 90 per cent of the firemen of acceptable age accepted the examination. Finally, a group of stevedores from the municipal international dock were examined and, again, most of those given the opportunity agreed to the examination.

Technic

Venous blood samples were drawn in the resting state without stasis and total cholesterol was measured in the serum by our modification25 of the method of Abell et al.36 In many samples total cholesterol was also measured in the alpha- and beta-lipoprotein fractions as separated by paper electrophoresis.35 All analyses were performed in duplicate. Identical methods were used in Minneapolis and in Naples and strict comparability of results in the two cities was assured by many exchanges of materials for analyses as well as exchanges of scientific personnel between the two laboratories.

Relative body weight was calculated from the recorded (nude) weight and (barefoot) height, by the use of standard tables for "average" weight at given height and age.28 We hold no brief for these tables as indicators of either the actual or the desirable body weight of the U. S. or any other population,32-34 but they do provide a generally available reference system in which individuals of differing height and age can be compared in terms of a single variable of relative weight.

Variability of Serum Cholesterol

All values reported here are subject to 2 sources of variation other than the variability among individuals. The first source, the error of the analytical measurement, is small, as shown by many series of replicate analyses. Elsewhere,17 we have reported a value of ± 3.61 mg. per 100 ml. for the standard error of measurement in 1,068 pairs of duplicate analyses using the present method in our Minnesota laboratory, the computation being (SEM)² = (ΣΔ²)/2N, in which Δ is the difference between duplicates and N is the number of pairs. Almost identical values for SEM were obtained in several series analyzed in the Naples laboratory. Recently our Minnesota laboratory participated in a cooperative study of variability of cholesterol measurement supervised by the Communicable Disease Center of the U. S. Public Health Service, duplicate serum samples being received in different batches for analysis blind. From the report from the statistical office of the Center we find that for analyses reported from our Minnesota laboratory the value was SEM = ±5.0, the slightly higher value than noted above being accounted for perhaps by the fact that in this test the duplicates were analyzed on different days and involved various technicians and equipment. In actual practice here the value of SEM is smaller than ±5.0 because, after the analyses of the duplicates are recorded, when any pair differs by as much as 20 mg. per 100 ml., the analyses are repeated on serum saved for the purpose.

The present study is based on the means of duplicate analyses in all cases so the error of analytical measurement, taken as the repeatability of the means of duplicates, must have been less than the values of SEM cited above and may be estimated as between 2.6 and 3.6 mg. per 100 ml. As will be seen below, this source of variability accounts for only a small fraction of the total intra-individual variability observed in the present study.

Analytical error in serum cholesterol estimation may be serious in many routine laboratories.37 But even in that study in which the standard error of measurement in commercial laboratories was many times what should be considered acceptable, it appeared that biologic variation is more important.

Intra-individual variability was estimated from the results of blood samples drawn from the same individuals on different occasions. Blood samples were drawn twice, at an interval of 2 to 6 weeks, from each of 60 clinically healthy, middle-aged men who were living and working as usual. The grand mean for these men was 244.4 and the standard error of measurement, calculated from the 60 pairs of mean values (or duplicates) was SEM = 18.1.

A special study was done on coronary patients from whom blood samples were drawn at monthly intervals for 4 consecutive months. All these patients suffered from angina pectoris and most of them had had one or more myocardial infarctions but none as recently as 3 months before the start of the study. These patients were under no special dietary control except that they were asked not to make conscious changes in their diets. At monthly interviews they reported that their diets were "about the same." No major changes in clinical status in the 30 patients retained for analysis here were noted during the 4-month period. It was considered that the intra-individual.

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variability observed in the 4 blood samples in this series should be fairly representative of non-hospitalized male coronary patients aged 40 to 66.

The results from this study are summarized in table 1. With 3 or more replications, \( \text{SEM}^2 = \frac{(T-T_1)^2}{N(k-1)} \), in which \( N = \text{number of individuals} \), \( k = \text{number of replications} \), \( T = \text{sum of the squares of all values on all individuals on all occasions} \), and \( T_1 \) is \( k/2 \) times the sum of the squared sums of all values for each individual. In order to see whether intra-individual variability is related to the mean cholesterol value of the individual, the 30 patients were divided into halves, the 15 patients with the lower mean cholesterol values and the 15 patients with higher values.

It appears that the more hypercholesteremic patients are much more variable than those with lower mean values, \( \text{SEM} \) being almost twice as great in the patient with a mean cholesterol value of 299.6 as in those with a mean of 232.3. But the data do not suggest any real difference between the patients and the healthy men other than that associated with the difference in mean cholesterol level. It appears that only from about 2 to 6 per cent of the total variance within individuals may be ascribed to analytical error, the remainder being biologic variability, including that of the diet.

**Recent versus Old Myocardial Infarcts**

The coronary patients in Naples were comprised of 38 men who had suffered a myocardial infarct within 30 days before examination and 70 men whose infarcts were older. Before a general analysis was attempted of the combined Neapolitan data, these two groups were compared. No significant difference was found between the means for age, relative body weight, or serum cholesterol concentration, the nearest approach to significance being in the last named variable. The mean serum cholesterol value of the men with recent infarcts was 11.4 mg. per 100 ml. higher than in those with old infarcts but the standard error of this difference was \( \pm 9.1 \), and the value of \( t \) of only 1.25 indicates that such a difference would be expected to occur by chance in about 1 out of 4 trials. Accordingly, general analysis was undertaken disregarding the age of the infarct.

**Results**

**Coronary versus Control Subjects**

The general results are summarized in table 2 with statistical analysis in table 3. Age matching between coronary and control subjects was satisfactory but in regard to both relative body weight and serum cholesterol concentration there were highly significant differences between groups.

In the Twin Cities there was obviously no difference in relative body weight between patients and control subjects and neither group contained a high frequency of overweight men. Among the 72 coronary patients there were only 12 men who were 10 per cent or more above the standard average relative body weight of the Medico-Actuarial tables and only 4 men were as much as 20 per cent overweight according to these criteria. In contrast, all of the Neapolitan groups except the working class control subjects tended to be overweight and within each Naples class the coronary patients tended more often to be overweight than the men in their control group though the latter difference was not significant for the upper class. Among the 54 working class Neapolitan coronary patients only 9 were as much as 10 per cent overweight but these included 5 men over 20 per cent overweight of whom one was a clerk with relative weight 145 who had had an infarct 2 months previously and who was the fattest man in the entire series of 751 men reported here.

The serum cholesterol trend is striking. As expected, both of the Twin Cities groups had significantly higher averages than either of the Neapolitan control series and the coronary patients in the Twin Cities differed from the Twin Cities control subjects with high significance. But even the Twin Cities control subjects had higher values than the Neapolitan coronary patients. Further, in each social class in Naples the patients had higher cholesterol averages than their control subjects. It is obvious that these data do not support the idea that there is a "normal serum choles-
Table 2
Means, and Standard Errors, for Relative Body Weight and Serum Total Cholesterol, in Men Aged Under 65 with Coronary Heart Disease and in Clinically Healthy Men Aged 40 to 65.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. men</th>
<th>Age</th>
<th>Relative body weight</th>
<th>Total cholesterol mg. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Twin Cities coronaries</td>
<td>72</td>
<td>52.8</td>
<td>98.7 ± 1.4</td>
<td>258.9 ± 4.1</td>
</tr>
<tr>
<td>Twin Cities controls</td>
<td>338</td>
<td>53.2</td>
<td>98.0 ± 0.9</td>
<td>238.9 ± 2.4</td>
</tr>
<tr>
<td>Naples upper class coronary patients</td>
<td>54</td>
<td>53.2</td>
<td>107.1 ± 1.4</td>
<td>217.6 ± 6.5</td>
</tr>
<tr>
<td>Naples upper class control subjects</td>
<td>89</td>
<td>50.3</td>
<td>104.5 ± 1.7</td>
<td>201.3 ± 5.1</td>
</tr>
<tr>
<td>Naples working class coronary patients</td>
<td>54</td>
<td>53.1</td>
<td>103.6 ± 1.5</td>
<td>203.7 ± 5.2</td>
</tr>
<tr>
<td>Naples working class control subjects</td>
<td>144</td>
<td>51.4</td>
<td>97.6 ± 1.4</td>
<td>170.7 ± 2.6</td>
</tr>
</tbody>
</table>

Table 3
Statistical Analysis of the Differences between the Several Groups of Patients and Control Subjects

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Degrees of freedom</th>
<th>Δ Relative weight</th>
<th>F</th>
<th>Δ Cholesterol</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. W. coronary minus T. W. control</td>
<td>408</td>
<td>0.7</td>
<td>—</td>
<td>20.0</td>
<td>13.06</td>
</tr>
<tr>
<td>T. W. coronary minus Naples I coronary</td>
<td>124</td>
<td>-8.4</td>
<td>17.14</td>
<td>41.3</td>
<td>31.40</td>
</tr>
<tr>
<td>T. W. coronary minus Naples II coronary</td>
<td>124</td>
<td>-4.9</td>
<td>5.26</td>
<td>55.2</td>
<td>71.66</td>
</tr>
<tr>
<td>Naples I coronary minus Naples II coronary</td>
<td>106</td>
<td>3.5</td>
<td>2.71</td>
<td>13.9</td>
<td>2.79</td>
</tr>
<tr>
<td>Naples I coronary minus Naples I control</td>
<td>141</td>
<td>2.6</td>
<td>1.14</td>
<td>16.3</td>
<td>3.87</td>
</tr>
<tr>
<td>Naples I control minus Naples II control</td>
<td>251</td>
<td>6.9</td>
<td>8.27</td>
<td>28.7</td>
<td>30.03</td>
</tr>
<tr>
<td>T. W. control minus Naples I control</td>
<td>425</td>
<td>6.5</td>
<td>11.07</td>
<td>37.6</td>
<td>49.27</td>
</tr>
<tr>
<td>Naples II coronary minus Naples II control</td>
<td>196</td>
<td>6.0</td>
<td>4.88</td>
<td>31.1</td>
<td>35.12</td>
</tr>
</tbody>
</table>

T. W. = Twin Cities, Naples I and Naples II designate upper and working classes, respectively. The probability values associated with the F values are 3.92, 6.85, and 11.38 for p = 0.05, 0.01 and 0.001, respectively, for 120 degrees of freedom (d.f.) and for d. f. = ∞ the corresponding F values are 3.84, 6.64, and 10.83.

terol” level for clinically healthy middle-aged men in general or that there is some kind of a critical level that distinguishes all or average coronary patients.

Analysis of the cholesterol data in terms of frequency distribution rather than mean values shows that the differences between the means are not due to a few extreme values. In the Twin Cities, 56 of 72, or 78 per cent, of the coronary patients had cholesterol values above the control median. Among the Naples coronaries 32 of 54 of the upper class patients were above the median of the upper class control and 44 of the 54 working class patients were above their control median.

The “working class” samples in Naples comprise men at the same general socioeconomic level who differed markedly in physical activity, at least prior to infarction. It is of interest, therefore, to compare the clerical workers with the men in occupations involving heavy manual labor as analyzed in table 4. The manual workers do not differ significantly from the clerical workers in relative body weight or in serum total cholesterol but in each of these activity categories the coronary patients tend to be relatively heavier and to have much higher serum cholesterol values than their controls of the same age and activity.

Alpha and Beta-Lipoprotein Cholesterol

In previous reports\textsuperscript{18, 19, 38-40} we have shown
that the serum total cholesterol differences among clinically healthy populations as well as the changes induced by dietary alterations are entirely accounted for by changes in the cholesterol concentration in the beta-lipoprotein fraction of the serum. Table 5 summarizes the findings on the serum separated into 2 fractions by paper electrophoresis.

The Twin Cities and the Naples Control Subjects differ greatly in beta-lipoprotein cholesterol but they have almost identical mean values for cholesterol in the alpha-lipoprotein fraction. This corresponds with all our previous findings in samples of clinically healthy men. The coronary patients, however, clearly tend to have lower alpha-lipoprotein cholesterol values both in the Twin Cities and in Naples and the averages of these two patient groups are not significantly different from each other. This corresponds with the reports of others that alpha-lipoprotein tends to be diminished in coronary patients.1,4,6,10,41 Elsewhere we have reported that alpha-lipoprotein cholesterol concentration in man is stable, in contrast with the beta fraction, in the face of dietary differences, both in controlled experiments18, 40, 41 and in populations.18, 19, 40

Relationship between Relative Weight and Serum Cholesterol

Previously we reported that among clinically healthy men of given age in restricted socioeconomic classes and cultures there is at most only a small correlation between relative obesity, or relative body weight, and serum cholesterol concentration. Table 6 summarizes the examination of this question with the coronary patients studied here. Each group of patients was classified in regard to relative body weight so we have 3 non-overlapping classes with approximately equal numbers of men in each. Table 6 gives the mean values for relative body weight and serum cholesterol for each of these classes.

In both socioeconomic classes in Naples the most underweight patients tend to have substantially lower serum cholesterol values than the rest of the patients in their class but the most overweight patients are no different in serum cholesterol from the patients with medium relative body weight. Among the Twin Cities patients there is no significant differ-

Table 4

<table>
<thead>
<tr>
<th>Group</th>
<th>No. men</th>
<th>Relative body weight</th>
<th>Cholesterol, Mg. % per 100 ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual workers, coronary patients</td>
<td>32</td>
<td>101.1 ± 1.5</td>
<td>202.3 ± 6.4</td>
</tr>
<tr>
<td>Manual workers, control subjects</td>
<td>109</td>
<td>97.7 ± 1.5</td>
<td>170.5 ± 3.0</td>
</tr>
<tr>
<td>Clerical workers, coronary patients</td>
<td>22</td>
<td>107.2 ± 5.2</td>
<td>205.8 ± 3.0</td>
</tr>
<tr>
<td>Clerical workers, control subjects</td>
<td>35</td>
<td>97.0 ± 2.1</td>
<td>171.3 ± 6.3</td>
</tr>
</tbody>
</table>

Table 5

<table>
<thead>
<tr>
<th>Group</th>
<th>No. men</th>
<th>$\alpha$ Cholesterol</th>
<th>$\beta$ Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Twin Cities coronary patients</td>
<td>60</td>
<td>37.4 ± 1.4</td>
<td>221.5</td>
</tr>
<tr>
<td>Twin Cities control subjects</td>
<td>332</td>
<td>41.0 ± 0.6</td>
<td>197.9</td>
</tr>
<tr>
<td>Naples coronary patients</td>
<td>56</td>
<td>34.4 ± 1.2</td>
<td>179.4</td>
</tr>
<tr>
<td>Naples control subjects</td>
<td>60</td>
<td>40.6 ± 1.2</td>
<td>155.0</td>
</tr>
</tbody>
</table>
ence in serum cholesterol among the three relative body weight groups.

It is obvious that if socioeconomic class were ignored, various relationships between relative body weight and serum cholesterol might be indicated, depending on the groups and the numbers in them. But such spurious relationships would be accounted for by the characteristics of the groups and not by any necessary real relationship between the 2 variables.

Twin Cities Coronary Patients in 1957 to 1959

During 1957 to 1959 Twin Cities coronary patients, referred by cooperating physicians as in 1953 to 1955, were studied. After omission of patients over 65, those whose referral was related to previous findings of peculiarities in serum cholesterol obtained elsewhere, and those who were following management programs of drugs and diet prescribed by their physicians to control cholesterol, there were 36 business and professional men judged to be comparable to the patients of 1953 to 1955 that are reported in this paper. From interviews it was found that 20 of the 36 men had made significant changes in their diets since diagnosis of the disease. The dietary changes involved reduction in calories or fats or both and had been made because of concern about the possible ill effects of high-fat and high-calorie diets. In most cases it appeared that a decision to make dietary changes stemmed from information from mass media, reinforced by discussions with friends and physicians, the latter, however, not going so far as to prescribe dietary alteration.

The average age of these 36 patients was 54.6 years and their average total cholesterol concentration was 246.5 mg. per 100 ml. of serum, with a standard error of ±8.0. This average is 13.4 mg. per 100 ml. lower than the average for the 1953 to 1955 series but it does not attain statistical significance because of the large variability in the 1957 to 1959 series.

Experience in the Twin Cities more recently (1959 to 1960) continues to show this trend, in which fewer and fewer patients seen a month or more after diagnosis of coronary heart disease are found who have not changed their diets or taken drugs to control the blood cholesterol level. While this may be laudable in many respects, it does mean that it becomes increasingly difficult to obtain valid samples of coronary patients in this region whose cholesterol values may be representative of the natural history of the disease.

Discussion

Intra-Individual Variability

Previously published analyses of variability in serum cholesterol concentration are not strictly comparable with the present material. The 33 men reported by Watkin et al.42 were elderly hospitalized patients living a monotonous life in a single institution on a diet stated to be "remarkably constant from day to day"; their variability cannot be accepted as representative of nonhospitalized men living "ad lib." Those patients, further, were considerably older (average about 70 years) and their cholesterol levels were much lower (average about 198 mg. per 100 ml.) than the men studied in the Twin Cities. Apparently the intra-individual variability of that group was somewhat lower than in any of the groups reported here in table 1 but would correspond roughly to the present data, if
consideration were given to the trend of variability to rise with the mean cholesterol level.

The study of cholesterol variability made by Thomas and Eisenberg48 concerned Johns Hopkins medical students who were much younger and had lower cholesterol values than the men in table 1. The Bloor method used by Thomas and Eisenberg was stated to be satisfactorily reproducible but their data on the standard error of measurement of duplicate analyses, computed as in the present paper, indicates an analytical error about twice as large as in our laboratory. The Bloor method used by Thomas and Eisenberg systematically gives values about 15 per cent higher than our method. This difference is caused by the fact that ester cholesterol gives a more intense color with the Lieberman-Burchard reagent than does free cholesterol. Free cholesterol is used as the standard, both in the Bloor and the present method, but in the Bloor method the ester cholesterol in the serum is not hydrolyzed to the free form as is done in our method.

The intra-individual variability observed by Thomas and Eisenberg is greater than found here. For young men studied at intervals of 12 to 18 months, values of SEM (as in our table 1) were 34.7, 27.3 and 37.4 mg. per 100 ml. (Bloor method) for groups with initial cholesterol values of 150 to 199, 200 to 249 and 250 to 299, respectively. In terms of the method used here, these SEM values would correspond to about 30, 23, and 32. In another series average values in units corresponding to our method of SEM = 24, 25, 23, and 24 were obtained for repetitions less than 5, 6, 7, and 12 months apart, respectively.

In men on uncontrolled diets, as in all the groups considered here, spontaneous dietary variations undoubtedly contribute to the intra-individual variability. For comparison with the data in table 1, we have the findings in 834 pairs of blood samples from middle-aged men on rigidly constant diets in an institutional situation where there was no possibility of supplementation or alteration of the individual diets.48 Each pair consisted of 2 samples drawn 1 to 2 weeks apart from 1 man. The value of SEM, calculated as in table 1, was ±11.52 and the grand mean of the 1,668 samples was 228.2 mg. of cholesterol per 100 ml. In another study with soldiers in the U.S. Army, similarly controlled, the SEM from 38 pairs of blood samples drawn 6 to 10 days apart was ±12.0 and the grand mean of the 76 samples was 209.7 mg. per 100 ml.48 The difference between these values and those in table 1 may be ascribed, at least in part, to the control of the diet and perhaps other stabilizing factors in a situation in which individual freedom is curtailed.

We conclude that something like half of the intra-individual variation observed in men in a stable state of physical health but living under ad lib. conditions possibly may be explained by variations in diet and associated circumstances. But the remaining variability is not explicable from this cause. Some investigators suggest that variations in emotion and stress are influential.44-47 In none of those studies, however, was there any real dietary control. Whatever may be the cause of this variability, allowance for it must be made in analyzing and interpreting data on serum cholesterol.

Intra-Individual Variability and Group Differences

These data on intra-individual variability provide a basis for estimating the effect of intra-individual variability in masking the real differences among groups of men. This is more easily seen in the hypothetical case in which we have a group of men whose individual true mean cholesterol values, which would be found by many repeated blood samplings, were all exactly the same. We now compare these men with another group, equally homogeneous but with a different common mean cholesterol value. Let us say the first group are coronary patients and their mean is 260 and the second are "healthy control subjects," with a mean of 240 mg. per 100 ml. For simplicity's sake, assume the same intra-individual variability in each group, represented by a value of SEM = 20, as in table 1.

In other words, the true mean value for the serum cholesterol concentration in each
man in the patient group is 260, but if many
blood samples were taken from any one pa-
tient, the distribution of cholesterol values of
these samples would show them spread out
around the mean of 260 so that about two
thirds of the values would be between 240
and 280, one sixth below 240, and one sixth
above 280. The distribution of the values in
single samples from all of the patients would
be the same, since each sample is presumably
a random sample from the patient concerned.
The distribution would be the same for the
healthy control subjects except it would cen-
ter on the mean of 240, and two thirds of the
values would fall within the range of 220 to
260.

If we have equal numbers, say 50, in each
of the 2 hypothetical groups we expect from
single blood samples from each man to find
the means of the groups to be approximately
260 and 240 and the grand mean to be close
to 250. With standard deviations of 20, the
expected standard errors of the 2 group
means would be about 20/\sqrt{50} = \pm 2.8 and
that of the difference between the 2 group
means would be about \pm 4. Hence we would
have \( t = \frac{20}{4} = 5 \) which indicates a highly
significant difference between the 2 groups.

This is very satisfactory but suppose we
ask the question, how sharply will a cutting
point of 250 separate the 2 groups when each
man is represented by a single blood sample?
The value 250 as a cutting point is \( \frac{(260-250)}{20} = 0.5 \) standard deviation units below the
mean of the coronary patients and an equal
amount above the control mean. From tables
of functions of the normal probability curve
this value of SD = 0.5 indicates that 38 per
cent of values for the patient group will be
included in the range 250 to 270, while 62
per cent of the values will fall outside these
limits, half being on the low side, half on the
high side. Accordingly, 31 per cent, or 15 to
16 patients will be expected to be recorded
as being below the cutting point and an equal
number of control subjects will be recorded
as above this point. In other words, about 31
of the 100 men will have overlapping values
in single samples and will be misclassified as
to true cholesterol level even though the true
mean value of every man in the patient group
is 20 mg. per cent higher than the true mean
of every man in the control group.

Critics of the hypothesis that serum choles-
terol concentration is important in the devel-
opment of atherosclerosis like to emphasize
the observed overlapping between the fre-
cquency distributions of cholesterol values in
coronary patients and in clinically healthy
controls. Being "clinically healthy" at a
given examination, however, is far from a
guarantee of freedom from serious athero-
sclerotic development; further, the factors
responsible for atherosclerotic development may
have ceased to operate long before the clinical
situation, perhaps triggered by other unrelated
factors, is notable. But aside from these seri-
sous complications, the masking effect of
intra-individual variability, noted above, must
be great. Actually, the values cited above
relate only to relatively short-time variability;
they must be considered as underestimates of
variability over a period of years.

**Cholesterol Values—Coronary versus Control
Subjects**

In view of the evidence presented here in
a comparison of relative body weights and
serum cholesterol values of coronary patients
in different populations, or in different socio-
economic classes, it must be ascertained that
the "controls" actually correspond to the
patients in all relevant variables save that of
coronary disease. An illustration of the dif-
fERENCE between matched controls and "con-
trols" without properly matching occupation,
ere., is seen in the study of Gertler, Garn, and
Lerman on young (under 40) coronary pa-
tients. The mean serum cholesterol value of
the patients was 286.5 \pm 6.6 and that of the
matched "controls" was 241.9 \pm 5.5. The
mean for the less carefully selected "controls"
was 224.4 \pm 3.5. The literature is replete with
eamples of the neglect in this regard. It is
not acceptable to compare patients and con-
trols who differ in socioeconomic status or in
which "controls" are patients "without evi-
dence of disease considered important in
cholesterol metabolism."

*Circulation, Volume XXII, December 1960*
Table 7

Summary of Comparisons of Men with Coronary Heart Disease with Probably Acceptable Controls in the Same Population

<table>
<thead>
<tr>
<th>Reference</th>
<th>Age</th>
<th>Control subjects N</th>
<th>Cholesterol</th>
<th>Coronary patients N</th>
<th>Cholesterol</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gertler, Garn, Lerman,⁹</td>
<td>40</td>
<td>97</td>
<td>242</td>
<td>97</td>
<td>237</td>
<td>45</td>
</tr>
<tr>
<td>Doyle et al.⁸</td>
<td>39-55</td>
<td>40</td>
<td>244</td>
<td>48</td>
<td>273</td>
<td>29</td>
</tr>
<tr>
<td>Lawry et al.⁹</td>
<td>40-49</td>
<td>686</td>
<td>240</td>
<td>70</td>
<td>275</td>
<td>35</td>
</tr>
<tr>
<td>Lawry et al.⁸</td>
<td>50-59</td>
<td>357</td>
<td>244</td>
<td>101</td>
<td>262</td>
<td>18</td>
</tr>
<tr>
<td>Lawry et al.⁹</td>
<td>60-69</td>
<td>117</td>
<td>235</td>
<td>66</td>
<td>255</td>
<td>20</td>
</tr>
<tr>
<td>Epstein et al.⁴⁰. Italo-American</td>
<td>40</td>
<td>215</td>
<td>221</td>
<td>17</td>
<td>235</td>
<td>14</td>
</tr>
<tr>
<td>Epstein et al.⁴⁰. Jewish-American</td>
<td>40</td>
<td>311</td>
<td>238</td>
<td>61</td>
<td>241</td>
<td>3</td>
</tr>
<tr>
<td>Nikkiila;¹ Keys et al.⁷⁰</td>
<td>40-62</td>
<td>158</td>
<td>265</td>
<td>26</td>
<td>281</td>
<td>16</td>
</tr>
<tr>
<td>Present, Twin Cities</td>
<td>40-65*</td>
<td>338</td>
<td>239</td>
<td>72</td>
<td>259</td>
<td>20</td>
</tr>
<tr>
<td>Present, Naples upper class</td>
<td>40-65</td>
<td>89</td>
<td>201</td>
<td>54</td>
<td>218</td>
<td>17</td>
</tr>
<tr>
<td>Present, Naples working class</td>
<td>40-65*</td>
<td>144</td>
<td>171</td>
<td>54</td>
<td>204</td>
<td>33</td>
</tr>
</tbody>
</table>

N, number of men; Cholesterol, serum total cholesterol mean, mg. per 100 ml. Ages matched except in the data from Epstein, Simpson, and Boas in which the mean age of coronary patients vs. control subjects was 59 vs. 56 for Italian men and 62 vs. 58 for Jewish men.

*Coronary patients include 2 men under 40 in each of these series.

Table 7 summarizes data comparing coronary patients with clinically healthy men who may be reasonably considered as controls, i.e., actually from the same population and corresponding, in general, in regard to socioeconomic status, etc. But none of the older series is free from cavil.

Some question may be raised in regard to the series reported by Lawry et al.⁸ which comprised men, largely self-selected, from many parts of the United States and who do not seem to have been deliberately matched in respect to region, occupation, etc. Though there is no proof of strict comparability in these regards, neither is there any evidence of bias. The series of Doyle et al.¹³-¹⁵ is confined to State of New York employees in one region but again it is not clear that the various kinds of occupation and socioeconomic status are equally represented in the coronary and the control series.

The series of Epstein et al.⁴⁸ is not open to question on these points; all were garment workers in New York City and the 2 groups were clearly distinguished in regard to ethnic origin. But these garment workers were largely in an older age range in which it is generally believed that the relationship of serum cholesterol level to the appearance of the clinical disease is less important than at younger years. Further, the coronary patients, especially the Jewish men, were older than their controls: 42 of 61 of the Jewish coronary patients were over 60 and 10 were over 70, and this proportion of old men was not represented among the control subjects. It should be noted that at ages over 60 the serum cholesterol level tends to fall, at least in clinically healthy men.¹¹, ⁴⁹, ⁵² It is probable that the exclusion of the aged coronary patients from the series of Epstein et al.⁴⁸ would result in a larger difference between coronary and control subjects.

In some investigations comparisons between coronary patients and control subjects have been published in a form that does not allow proper summary in table 7. The general nature of the findings in these is at least roughly in harmony with those summarized in table 7. In the Cooperative Study,¹¹ among the 57 men who subsequently developed coronary heart disease 41 (=72 per cent) had serum cholesterol values above the median for the population studied, the mean difference between those who did and those who did not subsequently exhibit the disease ranging from

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3.4 to 35.5 mg. of total cholesterol per 100 ml. of serum in the 5 subgroups, the grand average difference being 17.4. The 4-year follow-up of 898 men aged 45 to 63 in Framingham, Massachusetts, showed that the 52 new cases of coronary heart disease were distributed so that the new disease rate per 1,000 was 122 among men with serum cholesterol values of 260 or more on the initial examination, 45 among men with values of 225 to 259, and 40 among men with lower initial cholesterol values.12 In the study of Forssman and Lindegård50 of 51 coronary patients and 51 pair-matched control subjects the patients had the higher cholesterol values in 34 out of 51 pairs. Further, with 300 mg. of cholesterol per 100 ml. as the point of demarcation, 17 of the 51 patients but only 6 of the 51 control subjects were classed as having "hypercholesteremia."

In South Wales a prevalence study in the population in 2 selected regions included cholesterol measurements on 69 men judged to have "ischemic heart disease" and 205 others (control subjects), the diagnostic label being attached, apparently, on the basis of rather slight indications in most cases, judging from the very high prevalence rate reported.51 Details were not reported but it was stated that "the (cholesterol) difference (11 ± 14.6 mg. per 100 ml.) between those with and those without ischemic heart disease is small and statistically insignificant." From the standard error reported it would appear that these Welshmen must have been remarkably variable in regard to blood cholesterol. The average age of the coronary subjects, if they may be so termed, was about 60, i.e., somewhat older than the men of our primary concern here.

There are many other series that, unfortunately, cannot be included in table 7 because of lack of information or serious doubts about the control subjects. The series of Barr et al.1 provides only 21 control men aged 45 to 66, and these do not appear to have been matched to the coronary population in other respects. The several series of Oliver and Boyd8,6 provide age and sex matching of control and coronary subjects but the control subjects were made up of various noncoronary patients, medical doctors, etc., who scarcely represent the population from which the coronary patients were drawn. Nevertheless, these and other similar studies with questionable control groups agree in showing that coronary patients do tend to have relatively elevated serum cholesterol levels, especially at younger ages.

**Relative Body Weight—Coronary versus Control Subjects**

The situation in regard to relative body weight and obesity is equally complicated by failure to assure proper comparability in other respects of control subjects and patients. Because of variations in the methods of evaluating or reporting relative body weight or obesity, the findings in major papers are summarized in qualitative terms only in table 8. In 11 series in which the relative weight status of the patients was ascertained at the time of diagnosis or examination, the patients were not significantly more often overweight than their control subjects in 7 series involving 498 patients, but were more overweight in 4 series involving 237 patients. In 7 series in which evaluation of relative weight was based on data before the appearance of the disease, the patients were not more often overweight in 3 series, involving 968 patients and were more often overweight in 4 series involving 232 patients.

No clear trend emerges from these several studies except the conclusion that the frequency of gross overweight or obesity among coronary patients is not so high as is often thought. In some of the series in which relative overweight of the patients was evaluated at the time of examination or diagnosis it is possible that the finding of overweight underestimated the situation before the disease was first recognized because the patients may have reduced recently. This was not a prominent factor in the Twin Cities and Naples series reported here. On the other hand, in at least some of the series in which the coronary patients significantly tended to be more often overweight than their control subjects, it is
Table 8

Relative Body Weight of Coronary Patients Compared with Their Control Subjects

<table>
<thead>
<tr>
<th>Authors</th>
<th>No.</th>
<th>When seen</th>
<th>Patients, more often overweight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yater et al.(^a)</td>
<td>866</td>
<td>Pre-disease</td>
<td>No</td>
</tr>
<tr>
<td>Master et al.</td>
<td>100</td>
<td>Patients</td>
<td>Yes</td>
</tr>
<tr>
<td>Angina pectoris</td>
<td>100</td>
<td>Patients</td>
<td>No</td>
</tr>
<tr>
<td>Coronary occlusion</td>
<td>100</td>
<td>Pre-disease</td>
<td>Yes, recollection</td>
</tr>
<tr>
<td>Gertler, White and Others(^b)</td>
<td>97</td>
<td>Patients</td>
<td>No</td>
</tr>
<tr>
<td>Dawber et al.(^c)</td>
<td>52</td>
<td>Pre-disease</td>
<td>Yes</td>
</tr>
<tr>
<td>Doyle et al.(^d)</td>
<td>57</td>
<td>Pre-disease</td>
<td>Yes</td>
</tr>
<tr>
<td>Chapman et al.(^e)</td>
<td>13</td>
<td>Pre-disease</td>
<td>Yes</td>
</tr>
<tr>
<td>Ages 40-54</td>
<td>24</td>
<td>Pre-disease</td>
<td>No</td>
</tr>
<tr>
<td>Epstein et al.(^f)</td>
<td>17</td>
<td>Patients</td>
<td>Yes</td>
</tr>
<tr>
<td>Italo-Americans</td>
<td>61</td>
<td>Patients</td>
<td>No</td>
</tr>
<tr>
<td>Drake et al.(^g)</td>
<td>60</td>
<td>Patients</td>
<td>No*</td>
</tr>
<tr>
<td>Forasman and Lindegård(^h)</td>
<td>51</td>
<td>Patients</td>
<td>Yes</td>
</tr>
<tr>
<td>Buechley et al.(^i)</td>
<td>78</td>
<td>Pre-disease</td>
<td>No</td>
</tr>
<tr>
<td>Thomas et al.(^j)</td>
<td>69</td>
<td>Patients</td>
<td>Yes</td>
</tr>
<tr>
<td>Present, Twin Cities</td>
<td>72</td>
<td>Patients</td>
<td>No</td>
</tr>
<tr>
<td>Present, Naples upper class</td>
<td>54</td>
<td>Patients</td>
<td>? Not significant</td>
</tr>
<tr>
<td>Present, Naples working class</td>
<td>54</td>
<td>Patients</td>
<td>Yes</td>
</tr>
</tbody>
</table>

The column "When seen" indicates whether relative weight was measured as patients or from data some years before clinical disease ("pre-disease").

*Preliminary, from raw data.

It is obvious that at present it is increasingly difficult to find coronary heart disease patients after infarction in the Twin Cities who have not embarked on one or another regimen of diet and drugs in an effort to control serum cholesterol or body weight or, more frequently, both. This complication is beginning to appear also in Italy but the natural history of the disease at least in the Naples area is still infrequently masked from this cause. In other parts of the United States too it may be that patients usually suffer their disease without tampering with their diets but it appears probable that the time is coming when it will be difficult everywhere to find relatively young coronary patients, recovered from the acute phase after infarction, whose blood picture represents the situation in which the disease developed. During the acute phase the picture may be misleading. Dodds and Mills\(^i\) report that in many cases cholesterol metabolism is distorted in the acute phase after an infarction. The time may come when the only sure way to discover the long-time characteristics of coronary patients in regard to lipid metabolism will be from long-time prospective studies started when the men in the samples were clinically healthy.

We have no good data on the true frequency of coronary heart disease in any of the populations reported here but from all sources of evidence, including experience of a variety of surveys, hospital populations, etc., it seems clear that there is a marked difference between the Twin Cities and the 2 Neapolitan populations, the frequency of coronary heart disease at less than 2 Neapolitan populations, the frequency of coronary heart disease at less than than very advanced ages being most common in the Twin Cities and least common in the Naples working class, the Naples upper class being intermediate. Qualitatively, at least, the serum cholesterol averages seem to correspond with the population frequency of the disease.

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In each population the coronary patients tend to be drawn from the upper range of the distribution of cholesterol values in that population. But there is certainly no sign of a general critical level for the appearance of the disease. We may theorize that increasing levels of serum cholesterol are associated with increasing risk of the disease though other factors are contributory, perhaps to a major degree. If we have, then, populations with cholesterol frequency distributions differing as to means or other measures of the central tendency, we shall expect to find correspondingly different frequencies of the disease and average levels of serum cholesterol in the coronaries that also differ and are related to the characteristics of the populations in which they occur. This seems to be the situation with the 3 populations in the present study.

Relative body weight appears to play a less important role from these and other data but in some situations relative body weight and serum cholesterol are, or appear to be, interrelated. This interrelationship is most prominent when the population is heterogeneous and embraces large differences in socioeconomic status.

Summary

Men under 66 years of age suffering from unequivocal coronary heart disease in the Twin Cities of Minnesota and in Naples, Italy, were compared with clinically healthy men matched in place of residence, age, type of occupation, and socioeconomic status.

The Twin Cities patients \((N = 72)\) did not differ in relative body weight from their controls \((N = 338)\) and the average relative weight of both groups was less than either of the 2 Naples coronary groups and the upper class Naples control subjects. The average relative body weight of the upper class Naples coronary patients \((N = 54)\) was insignificantly greater than that of their controls \((N = 89)\). The Naples working class coronary patients \((N = 54)\) had a significant tendency to be more often overweight than their controls \((N = 144)\).

The serum total cholesterol concentration was significantly higher in each of the 3 groups of coronary patients than in their corresponding controls and the 3 groups, control and coronary, formed a sharp progression from high to low cholesterol values in the order: Twin Cities, Naples upper class, Naples working class. In the Naples working class, separation into manual and sedentary workers disclosed no differences in either relative body weight or serum cholesterol.

The differences in serum cholesterol were accounted for by cholesterol in beta-lipoprotein. None of the 3 control groups differed in alpha-lipoprotein cholesterol concentration but in each of the 3 populations the coronary patients tended to have lower alpha values than their controls.

The cholesterol data are compatible with the hypothesis that the 3 populations have different frequency distributions of cholesterol values, the coronary patients in each population tending to be drawn from the upper end of the corresponding population distribution, with no general critical level distinguishing all coronary patients as a total group.

In the past several years coronary patients in the Twin Cities tend to be so frequently on diets or drugs to control cholesterol that it is increasingly difficult to find patients who may properly characterize the natural history of the disease. The patients reported here were free from this complication.

Acknowledgment

We are grateful to Dr. Mario Mattioli in Naples and Drs. Reuben Berman and Ernst Simonson in Minneapolis for their aid in providing patients for study. Dr. Mario Maneini greatly aided the laboratory work in Naples. Cholesterol analyses in Minneapolis were carried out under the supervision of Dr. Joseph T. Anderson and Mrs. Nedra Foster.

Summario in Interlingua

Subjectos masculine de minus que 66 annos de etate qui sufrivea inequivocamentemente de morbo cardiac coro-nari in le "Citates Gemine" de St. Paul e Minne-apolis in Minnesota e in Neapole in Italia esseva comparate con clinicemente normal subjectos mascu-le de correspondente typos de domicilio, etates, occupationes, e stato socio-economic.

Le patientes in le Citates Gemine \((N=numero total =72)\) non differeva in le relative peso corpore ab le gruppo de controlo \((N=338)\), e le relative peso medie del duo gruppos esseva minus que illos in tanto
le un e le altere del duo gruopos coronari in Neapole como etiam le gruppo de controlo de classe socio-economic superior in Neapole. Le relative peso corpoere medie del patientes coronari de classe socio-economic superior in Neapole (N=54) esseva insignificantemente superior a illo del correspondente gruppo de controlo (N=89). Le patientes coronari de classe obrer in Neapole (N=54) tendeva significativamente a portar pesos excessive in comparation con le correspondente gruppo de controlo (N=144).

Le concentration seral de cholesterol total esseva significativamente plus alte in ambe le gruppos in le Citates Gemine que in tanto le un como etiam le altere gruppo de controlo in Neapole, e le patientes coronari in le Citates Gemine differeva in iste respecto con alte grados de significacion ab le subjectos de controlo in le Citates Gemine. Sed mesmo le subjectos de controlo in le Citates Gemine habeva plus alte valores que le patientes coronari in Neapole. Finalmente, in ambe le gruppos socio-economic in Neapole, le patientes habeva plus alte niveles medie de cholesterol que le correspondente subjectos de controlo. In le caso del classe obrer in Neapole, le separation in obreros manual e sedentari revelava nulle differentia in relative peso corpoere e nule in le nivello seral de cholesterol.

Le differentias del niveles de cholesterol seral esseva causante per le cholesterol in lipoproteina beta. Nulle del 3 gruopos de controlo differeva ab le altere 2 in le concentration del cholesterol de lipoproteina alpha, sed in cata un del 3 populationes le patientes coronari tendeva a exhibir plus basse valors alpha que le correspondente gruppos de controlo.

Le datos relative a cholesterol es compatibile con le hypothese que le 3 populationes ha differentia distributiones frequentional del valores de cholesterol e que in cata un del popolaziones le patientes coronari tende a pertiner in le area superior del correspondentie distributione de population, sin que un niello critic existe con validitate general pro le distinctione de omn le patientes coronari como gruppo total.

In le curso del passate annos, le patientes coronari in le Citates Gemine tende si frequentemente a viver sub regimes dietari o drogal visante a reducir lor niveles de cholesterol que il deveni de plus in plus difficile trovar patientes qui characterisa directemente le historia natural del morbo. Le patientes del presente reporto es exempte ab iste complication.

References
Immortality

A plant or vegetable consumed to ashes to a contemplative and school-Philosopher seems utterly destroyed, and the form to have taken his leave for ever; but to a sensible Artist the forms are not perished, but withdrawn into their incombustible part, where they lie secure from the action of that devouring element. This is made good by experience, which can from the Ashes of a Plant revive the plant, and from its cinders recall it into its stalk and leaves again. What the Art of man can do in these inferior pieces, what blasphemy is it to affirm the finger of GOD cannot do in these more perfect and sensible structures. This is that mystical Philosophy, from whence no true Scholar becomes an Atheist, but from the visible effects of nature grows up a real Divine, and beholds not in a dream, as Ezekiel, but in an ocular and visible object, the types of his resurrection.—Sir Thomas Browne. Religio Medici. Edited by W. A. Greenhill, M.D. London, Macmillan and Co., Ltd., 1950, p. 76.
Serum Cholesterol and Relative Body Weight of Coronary Patients in Different Populations
ANCEL KEYS and FLAMINIO FIDANZA

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