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# Grading Atherosclerosis in Aorta and Coronary Arteries Obtained at Autopsy

## Application of a Tested Method *

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*A method of assessing "atherosclerosis", if used according to certain rules, was shown in an earlier study to be capable of discriminating between groups of aortas or coronary arteries according to the quantity of certain defined lesions. It would not measure absolute amounts, but would show whether one group of specimens had more or less of the factor assessed than another and would indicate the statistical significance of this finding according to the number of specimens in each group.*

*The method has now been applied to a study of material from six communities in three countries.*

*This paper outlines how the rules of procedure were applied. Intra-observer and inter-observer calibration tests carried out in a routine manner during four "grading sessions" and inter-sessional tests are described.*

*The discriminatory power in comparing groups of specimens from nearly 3000 subjects is calculated and shown according to artery (thoracic aorta, descending aorta, right coronary, left anterior descending coronary, left circumflex coronary) and type of lesion ("total amount of atherosclerosis", "fatty streak", "fibrous plaque", "complicated lesion" and "calcification"). Observations on "coronary stenosis" were also made. The discriminatory power of the method was calculated for this factor and, contrary to many expectations, was found to be of practical value.*

*Definitions and general procedure are described in annexes.*

### INTRODUCTION

There have been several attempts in the last decade to assess atherosclerosis in human autopsy material and to relate the amount of atherosclerosis to some general characteristic—such as race, nationality, age, sex, cause of death, mode of life, geographical location, etc.—of the subjects from whom the specimens were obtained. The published results of these studies have been interesting and suggestive but usually not definite, either because the precision of the methods used to assess atherosclerosis has not been demonstrated or because the source of the autopsy material has not been representative of groups to be compared. These two difficulties are discussed below in the light of experience obtained by the authors while working as a research group on atherosclerosis; this research was co-ordinated by the World Health Organization.

#### *Precision of methods*

Methods used for assessing atherosclerosis are imprecise and the degree of imprecision must be measured and taken into account if definitive conclusions are to be drawn about differences found.

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To determine the extent of this difficulty and in the hopes of overcoming it a trial of methods of assessing atherosclerosis was organized by WHO during the years 1960-62. Arbitrary definitions of atherosclerosis were agreed and methods were adopted for assessing these factors. Some of these methods were found to be technically impracticable. After preliminary study, improved definitions, criteria and techniques were developed and specimens of aortas and coronary arteries were prepared to test four methods. After trial by 14 pathologists in five laboratories, it was found that one of these methods—that described by Holman et al.[1] and used in the International Atherosclerosis Project (PIA) was likely to be of practical use in that, though crude, it was likely, after its imprecision was taken into account, to produce statistically valid results with as few as 50 specimens from each group to be compared.[2]

On the basis of this trial and in conjunction with colleagues in the PIA a working procedure—called the Standard Operating Protocol—was drawn up. It has much in common with a similar protocol developed by PIA. It incorporates definitions, methodology and a procedure for ensuring comparability of data, together with details of the preparation of specimens, and is published as Annex 1 to this paper.

Briefly, it may be stated here that the specimens of aortas and coronary arteries, and data about their source, are collected in a prescribed manner. The specimens are then identified by number only, prepared in a standard manner at a central laboratory in Malmö, Sweden, and graded from time to time by a group of pathologists according to prescribed definitions and criteria.[3] It is essential that the graders do not know the source of any specimen, that all receive an equal proportion of specimens from each source, and that certain calibration tests described below are carried out during the grading procedure.

*Representative sample*

The second difficulty has been with the source of the material. Thus, if more atherosclerosis is found in material from males than from females or from one community than another, before conclusions can be drawn about differences in males and females or communities it is necessary to show that the material on which the conclusions are based comes from all, or a representative sample of all, of the males and females, or the community, etc. In practice this has not been so. The material is obtained from hospital or forensic autopsies. Generally, such material represents no more than about half the deaths, and the factors which determine whether a death will be autopsied or not are complex and vary from one community to another. These factors may influence the autopsy findings and therefore invalidate any conclusions extrapolated to groups or communities.

A true representative sample of the dead, sick and living of a community obviously cannot be studied at autopsy. It is in the hope that forensic death brings a random sample of the community to autopsy that these have been selected by some investigators for study. Unfortunately we found that in different communities there were different criteria of forensic death, that different proportions of these deaths (usually less than half) and different sections of them were autopsied, and on the whole that relatively small numbers of autopsies were obtained. Additional information such as age, occupation, and medical history was often unobtainable in such cases.

To overcome this difficulty well defined communities were sought in which a high proportion of all deaths could be autopsied. It would then be possible to compare pathology in the dead by community, age, sex, cause of death, etc. Furthermore, providing changes did not take place rapidly in the populations, it could be predicted that certain identifiable groups of living communities would give rise to deaths of a characteristic group pathology in a predictable time. Under these circumstances, studies of the living groups could be usefully sandwiched between autopsy studies. The original autopsy studies would indicate which living groups to study and deaths arising from these groups would subsequently be autopsied.

## APPLICATION

*The sample*

By the commencement of 1963 the group, by studying autopsy and mortality records by age and sex, had identified seven communities in which 80%-90% of all deaths, of a demographically defined section of the community, would be likely to be available at autopsy. It seemed likely that, with

[1] Holman, R. L., McGill, H. C., Jr, Strong, J. P. & Geer, J. C. (1958) *Lab. Invest.*, **7**, 42-47.

[2] Kagan, A. & Uemura, K. (1962) *Bull. Wld Hlth Org.*, **27**, 667-679.

[3] The grading procedure is described in the Standard Operating Protocol (see Annex 1).

special attention. the proportion of deaths autopsied would be nearer 90% than 80% and that some information—e.g., on age, sex, and often occupation and "death certificate cause of death"—would be available in those cases where autopsy could not be done.

*Communities studied*

One of the seven communities was unable to take part in the group study that was planned. From the other six communities—Malmö, Sweden; Prague (Area II), Czechoslovakia; Ryazan, USSR; Tallin, USSR; Tartu, USSR; and Yalta, USSR—specimens have been collected from January 1963 onwards from about 6000 subjects and half of these (i.e., material from some 3000 subjects) have been graded, in four sessions, with respect to atherosclerosis in the aorta and corony arteries. Some conclusions based on the study so far will be reported in a later paper.

*Precision of methods*

Here we report the steps taken to ensure that assessment of atherosclerosis in vessels from different sources by several pathologists, and on several occasions could be compared. The precision and discriminatory power found during the first four grading sessions is also shown. Definitions and criteria adopted and procedures taken to ensure comparability of data—such as preparation of the specimens, recording of basic data, separation of specimens from basic data, etc.—are described in the Standard Operating Protocol (see Annex 1),

Variation that requires to be reduced and assessed is due to inter-observer differences in the interpretation of definitions and criteria during a particular grading session, intra-observer variation in the application of such definitions and criteria during a particular grading session, and group variation of these factors between grading sessions.

*Standard specimens*

In order to maintain continuously the level of conformity of grading and also to train new graders, a set of "standard" specimens has been selected. For these purposes, the specimens are sent to participants for study between grading sessions. The "standard" set represents the range of types of material to be encountered in grading sessions and, in particular, includes specimens giving rise to special difficulty (e.g., definition of lesions).

*Inter-observer differences in interpretation of definitions and criteria during a particular grading session*

Before the grading of specimens commences, a few specimens representing the range of factors to be assessed are given to the graders. Each grader is equipped with a copy of the definitions and criteria and each independently grades all the specimens. The results are then compared and, where large differences in grading are found, the specimens are re-examined and the reasons for the disparity discussed. In this way differences in interpretation are disclosed, discussed and reduced. The results of this preparatory session are not taken into account in the study proper. Differences still existing between graders are neutralized with respect to the source of the specimens during each grading session by distributing specimens from each source equally among the graders.

*Intra-observer variation in the application of definitions and criteria during a particular grading session*

In the study as designed this is the most important cause of lack of comparability between data obtained during a particular grading session. It is reduced by clarification of definitions and criteria and by practice. It is assessed by presenting to each grader specimens that he has already graded. The difference between the first and second measurements is noted. In order to ensure some control over the whole of the grading session each day's grading is divided into quarter sessions and each quarter session (except the first) includes a few repeat specimens from a previous quarter session. Fig. 1 shows in diagrammatic form how test specimens are circulated for the assessment of the intra- and inter-observer variations in the grading session.[1]

*Inter-session variation*

This is measured so that data obtained at different grading sessions may be compared. Some of the specimens seen at a previous grading session are included for re-examination.

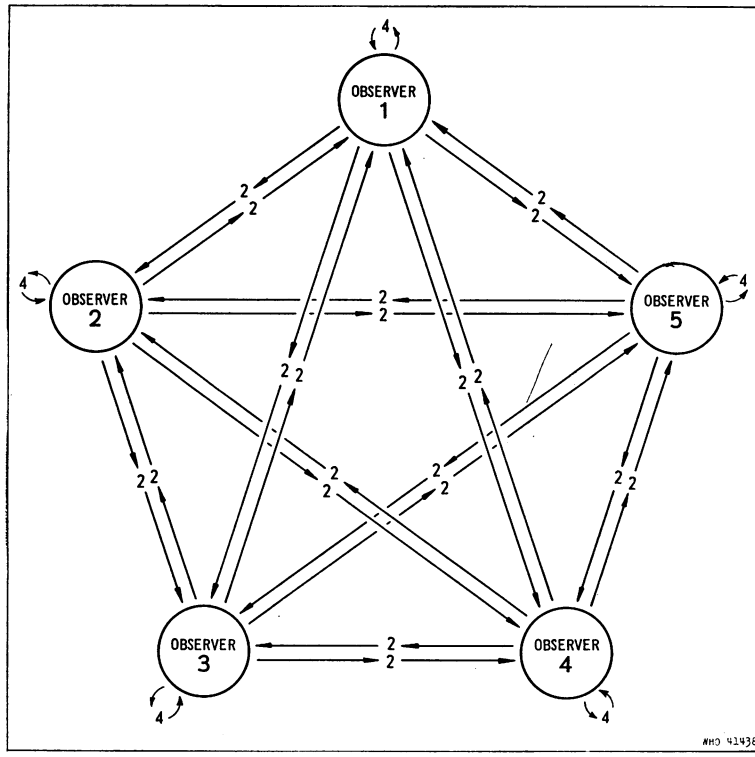
## RESULTS

*Source of material*

Table 1 shows the total number of deaths and the number autopsied and included in the study for Malmö, Prague II, and Yalta in 1963; Table 2 gives similar information for Tallin and Ryazan.

[1] A table has been prepared showing in full detail how the intra-observer, inter-observer and inter-session comparability tests are conducted. This has been deposited in the WHO Library, from which copies may be obtained on request.

FIG. 1
A SCHEME FOR CONDUCT OF INTRA- AND INTER-OBSERVER COMPARABILITY TESTS [a]




[a] In the intra-observer test, 4 specimens are to be graded twice by the same observer. In the inter-observer test, 2 specimens graded by observer 1 are to be passed to observer 2; another pair of specimens is to be passed to observer 3; another pair to observer 4; and another to observer 5. Similar exchanges are to be made from observer 2 to observers 1, 3, 4 and 5; and so on.

For Malmö the number of autopsies in the study was very little less than the number of deaths and approximates to 80%-90% for both sexes from 40 to 79 years of age. In Prague II approximately 90% of the deaths were autopsied but only 70% came into the study. This is because in the first part of the year a high proportion of deaths autopsied away from the main centre were not regarded as part of the study until it was too late to include them. In Yalta a high proportion of deaths came into the study up to the age of 59 and in Tallin and Ryazan to the age of 49; deaths in later years frequently occur at home.[1] Autopsy in such cases is likely to be available in the future. Data from Tartu are not yet available.

*Precision of methods*

The results are shown separately for intra-observer and inter-session comparability tests for aorta and coronary arteries and for the different categories of atherosclerosis—total amount, fatty streak, fibrous plaque, complicated lesion, calcification.[2] In addition, assessment of stenosis[2] in coronary arteries has been studied.

[1] In one community a special effort was made to obtain autopsy on deaths occurring at home. Through co-operation between local authorities, general practitioners, the University department and relatives, this proved to be a success. In the first three months more than half the home deaths were autopsied and in the second three months over 95%. The general practitioners were very satisfied with the arrangement, which enabled them to receive a detailed autopsy report and to take part in the epidemiological study. In spite of the fact that home deaths were only a small proportion of all deaths in this community, it is mentioned here because hitherto it has been said that it is impossible to autopsy such deaths and their pathology has remained somewhat mysterious. The success in this community has encouraged others in the group and may encourage others outside it.

[2] See definitions in Annex 1 (page 310).

TABLE 1

PROPORTION OF DEATHS INCLUDED IN THE AUTOPSY STUDY, BY AGE-GROUPS, IN MALMÖ, PRAGUE II AND YALTA, 1963

| Age-group (years) | Sex | Malmö | | | Prague II | | | Yalta | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Total deaths | Autopsied and studied | | Total deaths | Autopsied and studied | | Total deaths | Autopsied and studied | |
| | | | No. | % | | No. | %[a] | | No. | % |
| 10-19 | M | 6 | 4 | | 5 | — | | — | — | |
| | F | 6 | 4 | | 8 | — | | 1 | 1 | |
| | M+F | 12 | 8 | 67 | 13 | — | 0 (77) | 1 | 1 | 100 |
| 20-29 | M | 14 | 9 | | 8 | 6 | | 10 | 10 | |
| | F | 9 | 7 | | 2 | 1 | | 8 | 7 | |
| | M+F | 23 | 16 | 70 | 10 | 7 | 70 (100) | 18 | 17 | 94 |
| 30-39 | M | 19 | 10 | | 8 | 3 | | 27 | 24 | |
| | F | 15 | 13 | | 8 | 5 | | 12 | 8 | |
| | M+F | 34 | 23 | 68 | 16 | 8 | 50 (88) | 39 | 32 | 82 |
| 40-49 | M | 56 | 52 | | 22 | 15 | | 23 | 20 | |
| | F | 42 | 40 | | 9 | 7 | | 13 | 10 | |
| | M+F | 98 | 92 | 94 | 31 | 22 | 71 (94) | 36 | 30 | 83 |
| 50-59 | M | 140 | 119 | | 91 | 59 | | 35 | 25 | |
| | F | 87 | 76 | | 59 | 37 | | 31 | 25 | |
| | M+F | 227 | 195 | 86 | 150 | 96 | 64 (91) | 66 | 50 | 76 |
| 60-69 | M | 230 | 196 | | 162 | 129 | | 31 | 24 | |
| | F | 166 | 143 | | 132 | 95 | | 42 | 20 | |
| | M+F | 396 | 339 | 86 | 294 | 224 | 76 (92) | 73 | 44 | 60 |
| 70-79 | M | 293 | 231 | | 177 | 124 | | 26 | 8 | |
| | F | 280 | 221 | | 216 | 162 | | 53 | 17 | |
| | M+F | 573 | 452 | 79 | 393 | 286 | 73 (88) | 79 | 25 | 32 |
| 80+ | M | 298 | 188 | | 74 | 56 | | 18 | 2 | |
| | F | 354 | 206 | | 158 | 118 | | 35 | 5 | |
| | M+F | 652 | 394 | 60 | 232 | 174 | 75 (81) | 53 | 7 | 13 |
| Total | M | 1 056 | 809 | | 547 | 392 | | 170 | 113 | |
| | F | 959 | 710 | | 592 | 425 | | 195 | 93 | |
| | M+F | 2 015 | 1 519 | 75.4 | 1 139 | 817 | 71.7 (88.1) | 365 | 206 | 56.4 |

[a] Figures in parentheses indicate the autopsy rate (%) in Prague II, including those cases from which no specimens of aorta and coronary arteries were collected, i.e., not included in the study.

TABLE 2
PROPORTION OF DEATHS INCLUDED IN THE AUTOPSY STUDY, BY AGE-GROUPS, IN TALLIN AND RYAZAN, 1963

| Age-group (years) | Sex | Tallin | | | Ryazan | | |
|---|---|---|---|---|---|---|---|
| | | Total deaths | Autopsied and studied | | Total deaths | Autopsied and studied | |
| | | | No. | % | | No. | % |
| 10-19 | M | 25 | 21 | | 22 | 18 | |
| | F | 7 | 7 | | 15 | 11 | |
| | M + F | 32 | 28 | 87.5 | 37 | 29 | 78.4 |
| 20-29 | M | 64 | 63 | | 78 | 62 | |
| | F | 16 | 15 | | 27 | 21 | |
| | M + F | 80 | 78 | 97.5 | 105 | 83 | 79.0 |
| 30-39 | M | 81 | 74 | | 123 | 105 | |
| | F | 31 | 22 | | 40 | 29 | |
| | M + F | 112 | 96 | 85.7 | 163 | 134 | 82.2 |
| 40-49 | M | 84 | 75 | | 102 | 76 | |
| | F | 53 | 34 | | 46 | 34 | |
| | M + F | 137 | 109 | 79.6 | 148 | 110 | 74.3 |
| 50-59 | M | 163 | 123 | | 154 | 104 | |
| | F | 129 | 83 | | 80 | 43 | |
| | M + F | 292 | 206 | 70.5 | 234 | 147 | 62.8 |
| 60-69 | M | 214 | 118 | | 142 | 58 | |
| | F | 282 | 114 | | 118 | 28 | |
| | M + F | 496 | 232 | 46.8 | 260 | 86 | 33.1 |
| Total | M | 631 | 474 | | 621 | 423 | |
| | F | 518 | 275 | | 326 | 166 | |
| | M + F | 1 149 | 749 | 65.2 | 947 | 589 | 62.2 |

The results of intra-observer comparability tests are summarized in Table 3. The average differences between each observer's first and second gradings have been computed at each session and averaged over the four grading sessions so far held. Since the number of cases graded at each session varied, a weighted average was computed by assigning the result of each session a weight proportionate to the number of cases graded.

The findings at the four grading sessions show a comparable degree of intra-observer variation. However, there appears to be some gradual improvements in the grading of the coronary arteries at each session. The intra-observer variation in assessing coronary stenosis was somewhat higher in the second session than in the other sessions. This was mainly due to a new grader, who participated in the second session for the first time.[1]

The frequency distribution of the intra-observer difference is shown in Fig. 2 and 3 for aortic and coronary atherosclerosis respectively.

[1] The steady improvement shown was continued into the subsequent grading sessions.

TABLE 3

AVERAGE INTRA-OBSERVER VARIATION (%) [a] MEASURED AT EACH GRADING SESSION

| Artery | Grading session | Total amount (%) | Fatty streak (%) | Fibrous plaque (%) | Complicated lesion (%) | Calcification (%) | Stenosis [b] (%) |
|---|---|---|---|---|---|---|---|
| Aorta | 1 | 5 | 6 | 6 | 3 | 4 | |
| | 2 | 7 | 5 | 9 | 2 | 3 | |
| | 3 | 7 | 5 | 10 | 4 | 4 | |
| | 4 | 6 | 5 | 8 | 2 | 2 | |
| | Average [c] | 6.4 | 4.9 | 8.9 | 2.7 | 3.0 | |
| Coronary | 1 | 10 | 6 | 13 | 2 | 6 | 7 |
| | 2 | 7 | 6 | 11 | 0.4 | 5 | 17 |
| | 3 | 8 | 5 | 11 | 0.8 | 3 | 5 |
| | 4 | 7 | 3 | 9 | 0.7 | 2 | 6 |
| | Average [c] | 7.4 | 4.1 | 10.1 | 0.7 | 3.2 | 8.7 |

[a] The unit taken is the percentage of total intimal surface area estimated to be involved.

[b] [Disagreement rate with respect to the presence or absence of coronary stenosis, i.e., $\frac{\text{Number of disagreements}}{\text{Total number of comparisons}} \times 100\%$.

[c] The result at each grading session was weighted by the number of cases graded, i.e., 100 at the first, 800 at the second, 1000 at the third and 1100 at the fourth session.

In a comparison of the graded amount of lesion between groups of specimens the intra-observer variation is not the only source of variation. There exist also intrinsic variations from one specimen to another within any group. The variability that is observed in the graded results is a combination of the variability arising from these two sources. The magnitude of the total variation has been studied in groups according to age, sex and broad causes of death. This is compared in Table 4 with the intra-observer variance computed from the data presented in Fig. 2 and 3. It is seen that the intra-observer variance constitutes about 8%-29% of the total variability.

The intra-observer variance is relatively large in the assessment of complicated lesions in the aorta and for fatty streaks, fibrous plaques and complicated lesions in the coronary arteries. On the

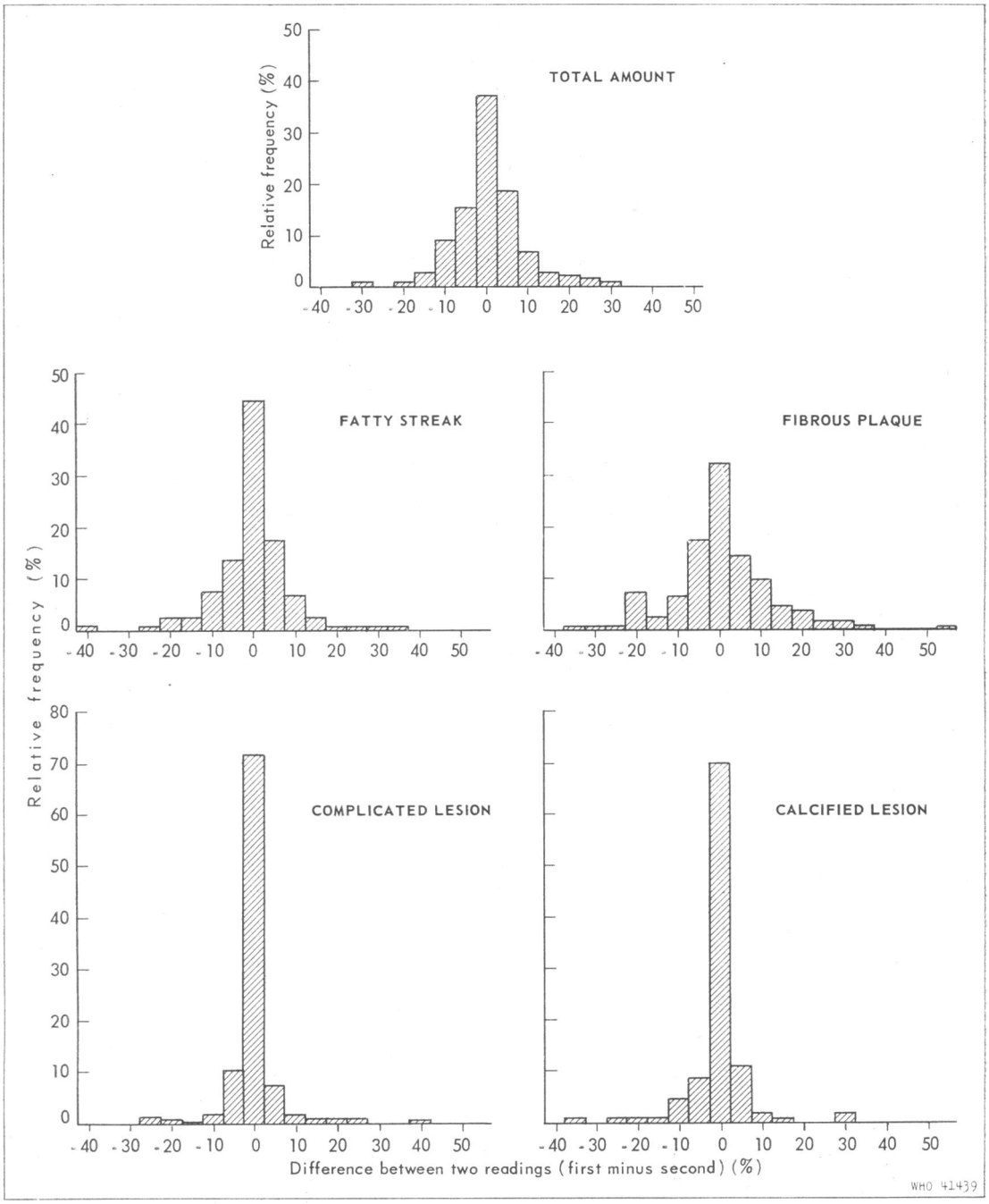
TABLE 4

MAGNITUDE OF INTRA-OBSERVER VARIATION RELATIVE TO THE TOTAL VARIANCE IN THE GRADED RESULTS

| Type of lesion | Aortas | | | Coronary arteries | | |
|---|---|---|---|---|---|---|
| | Total variance | Intra-observer variance | | Total variance | Intra-observer variance | |
| | | Variance | % of total | | Variance | % of total |
| Total amount | 475 | 40 | 8 | 727 | 86 | 12 |
| Fatty streak | 173 | 34 | 20 | 100 | 23 | 23 |
| Fibrous plaque | 444 | 73 | 16 | 553 | 126 | 23 |
| Complicated | 84 | 21 | 25 | 14 | 4.0 | 29 |
| Calcified | 120 | 21 | 18 | 176 | 33 | 19 |

FIG. 2

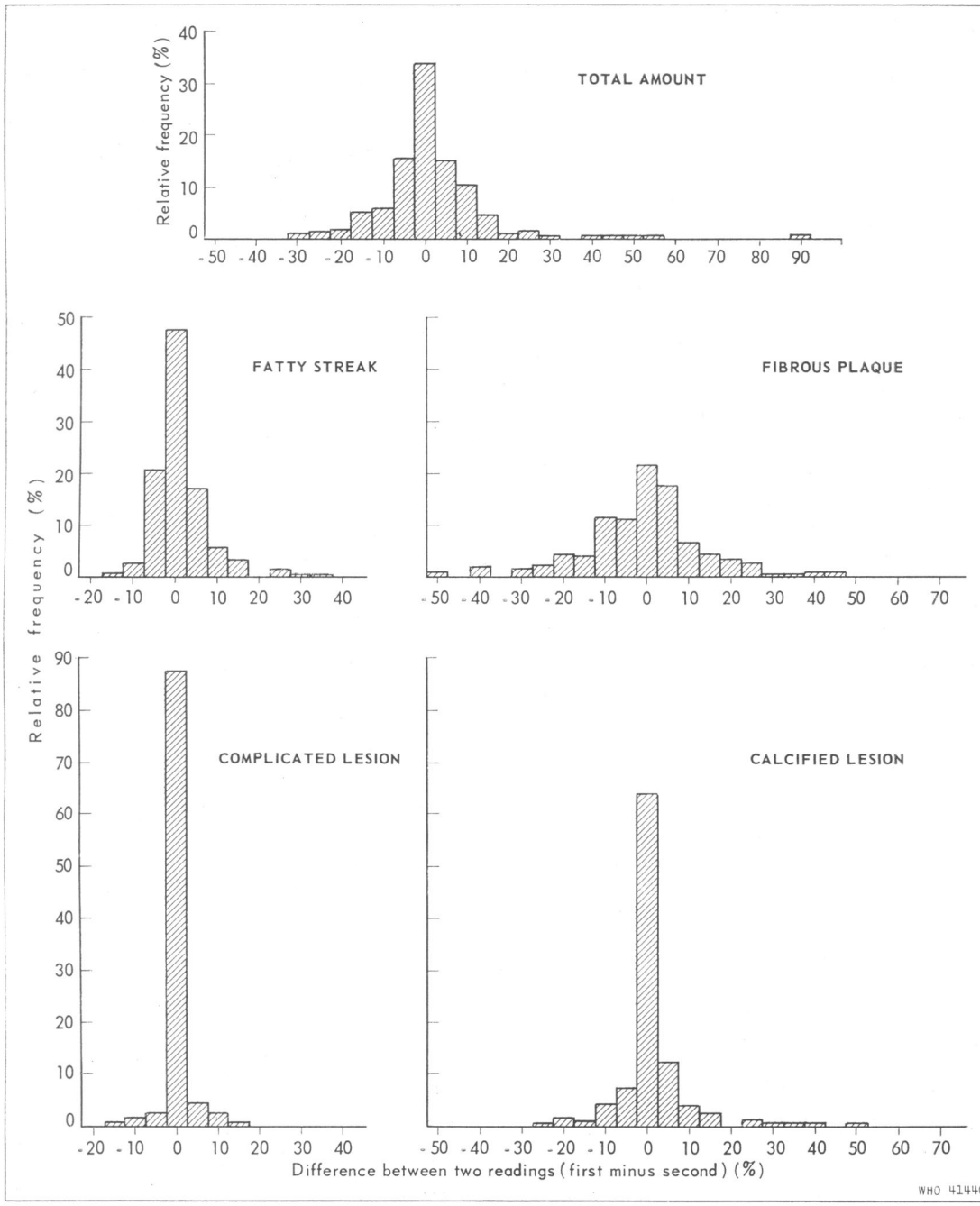
FREQUENCY DISTRIBUTION OF THE INTRA-OBSERVER VARIATION IN GRADING AORTIC ATHEROSCLEROSIS [a]




[a] Total number of comparisons: 210.

FIG. 3

FREQUENCY DISTRIBUTION OF THE INTRA-OBSERVER VARIATION IN GRADING CORONARY ATHEROSCLEROSIS[a]




[a] Total number of comparisons: 205 or 206.

FIG. 4
OBSERVED STATISTICALLY SIGNIFICANT DIFFERENCES (P = 0.05)
IN THE AMOUNT OF AORTIC ATHEROSCLEROSIS BETWEEN TWO GROUPS

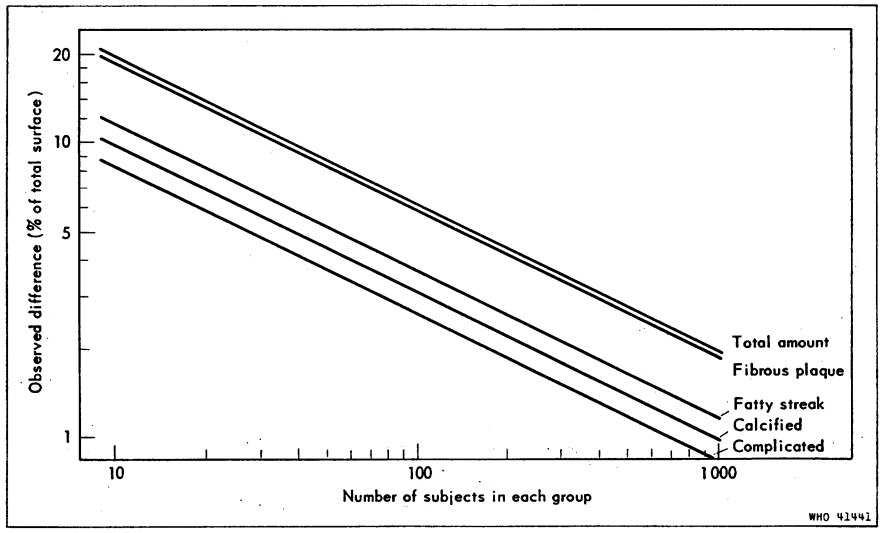



FIG. 5
OBSERVED STATISTICALLY SIGNIFICANT DIFFERENCES (P = 0.05)
IN THE AMOUNT OF CORONARY ATHEROSCLEROSIS BETWEEN TWO GROUPS

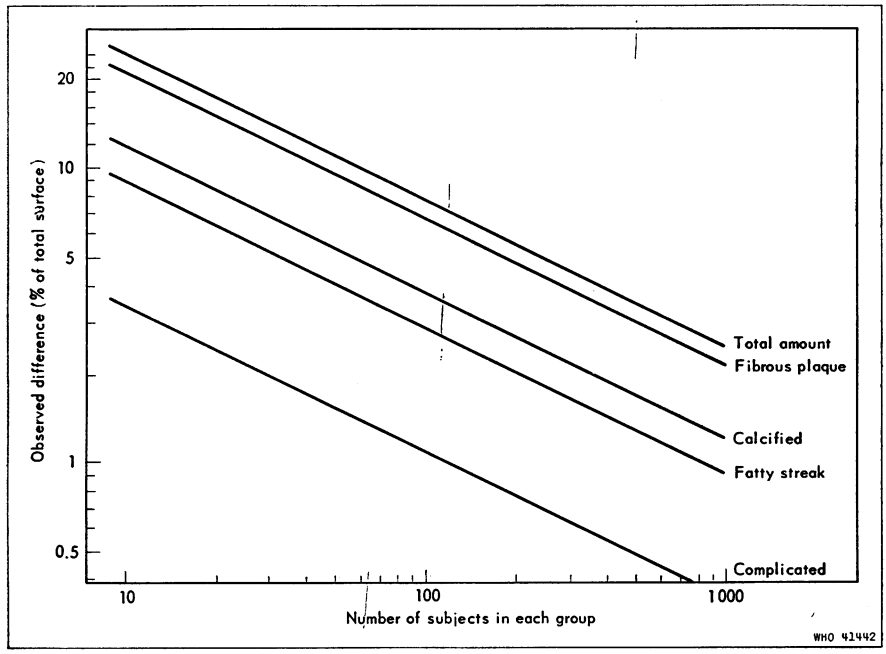
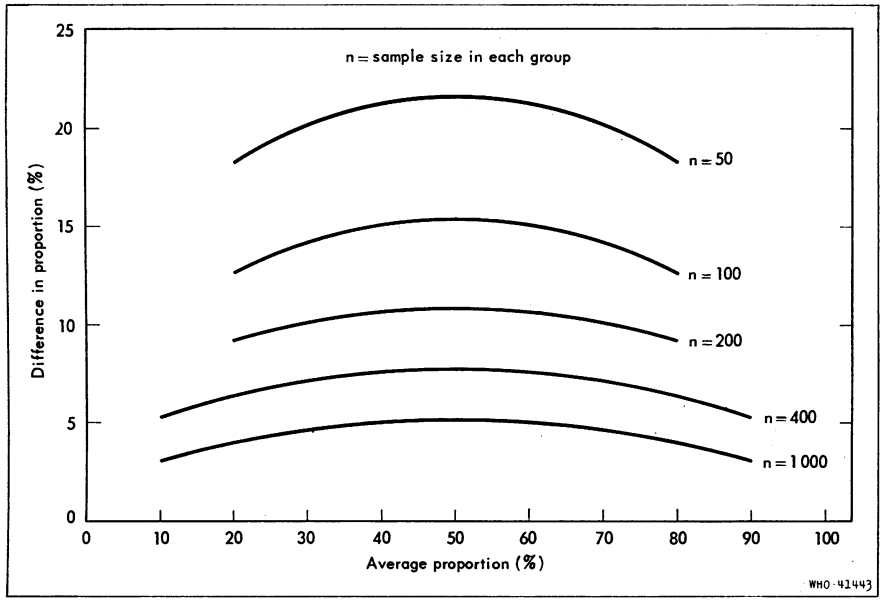


FIG. 6
OBSERVED STATISTICALLY SIGNIFICANT DIFFERENCES (P = 0.05)
IN THE PROPORTION OF CORONARY STENOSIS BETWEEN TWO GROUPS




contrary, the influence of the intra-observer variance is relatively small for the total amount of atherosclerosis in both aorta and coronary arteries.

Two charts (Fig. 4 and 5) have been constructed on the basis of Table 4, indicating broadly the magnitude of difference in assessment of total amount of atherosclerosis, fatty streak, fibrous plaque, complicated lesion and calcified lesion, which should be regarded as statistically significant (P = 0.05) in group comparisons. However, strictly speaking, the degree of variability depends on the groups to be studied, and a more precise test can be applied in each specific situation on the basis of the variability actually observed in the groups. The purpose of the two charts is merely to give an idea of the discriminatory power of the method as demonstrated in the grading sessions so far held. Fig. 6 gives similar information for coronary stenosis.[1]

Reference to Fig. 4 shows that under the conditions of the study so far the method permits of discrimination (at the 5% significance level) between two groups of 50 aortas in which the difference found for each condition exceeds the following percentage:

| | |
|---|---|
| Total amount of atherosclerosis | 9% |
| Fibrous plaque | 9% |
| Fatty streak | 6% |
| Calcified lesion | 5% |
| Complicated lesion | 4% |

For coronary arteries (Fig. 5) significant differences between two groups of 50 arteries would be as follows:

| | |
|---|---|
| Total amount of atherosclerosis | 11% |
| Fibrous plaque | 10% |
| Calcified lesion | 6% |
| Fatty streak | 4% |
| Complicated lesion | 2% |

Fig. 6 shows that for two groups of 50 coronary arteries a finding of a difference of 18%-21% or more in prevalence of stenosis (according to the average level of prevalence) would indicate a statistically significant difference at the 5% level of probability. With two groups of 100 arteries or more the discriminating difference would be 12%-15% or more.

[1] In the construction of Fig. 6 the total variance has been assumed to be equal to the sum of two binomial variances—(1) variance due to sampling, which depends on the stenosis rate itself; and (2) intra-observer variance (with P = 0.087/2 = 0.044, where the value 0.087 is the disagreement rate between two readings on the same specimen). The normal approximation has been used.

TABLE 5

AVERAGE INTER-SESSION VARIATION (%) [a] BASED ON RESULTS AT GRADING SESSION 2

| Artery | Grading session | Total amount (%) | Fatty streak (%) | Fibrous plaque (%) | Complicated lesion (%) | Calcification (%) | Stenosis [b] (%) |
|---|---|---|---|---|---|---|---|
| Aorta | 1 | 0 | −2 | −3 | +6 [c] | −2 | |
| | 2 | 0 | 0 | 0 | 0 | 0 | |
| | 3 | +4 [c] | −1 | +6 [c] | 0 | −1 | |
| | 4 | +4 [c] | −2 | +10 [c] | −2 | −2 | |
| Coronary | 1 | −1 | +2 | 0 | −1 | +2 | (−12) [d] |
| | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 3 | +1 | −1 | +3 | 0 | −2 | −5 |
| | 4 | +3 | −3 [c] | +7 [c] | 0 | −1 | +5 |

[a] The unit taken for all except coronary stenosis is the percentage of total intimal surface area estimated to be involved.
[b] The unit taken is the cases with stenosis as a percentage of total cases examined.
[c] Statistically significant discrepancies ($P < 0.01$).
[d] Based on 17 comparisons only.

In the evaluation of the inter-session discrepancies the results of the second grading session were taken as the base instead of those of the first session because, at the first session, only a small number of specimens was graded. Summary results are presented in Table 5. Figures with the positive sign (+) indicate a higher grade and those with the negative sign (−) a lower grade than in the second session. A few of the discrepancies are statistically significant; although the discrepancies are not large, they indicate the need for a continuous effort to ensure uniformity of grading procedure through different grading sessions.

## CONCLUSIONS

Conclusions based on the results of this study to date will be more fully reported in a later paper. Here it may suffice to say the following.

The intra-observer variation found varies for different factors but generally does not exceed 10%. This applies to the assessment of coronary stenosis (as defined) as well.

The inter-sessional variation is of the order of 5%.

The order of discriminatory power of the method in practice has been demonstrated, i.e., a difference of less than 15% found between two groups of specimens reflects a real difference ($P = 0.05$) with as few as 50 specimens from each group for the various defined types of atherosclerosis and with 100 specimens from each group for coronary stenosis. This is an improvement on the forecast from the original trial.[1]

The method as applied permits an assessment of its power to discriminate between amounts of atherosclerosis in two or more groups of specimens, in turn permitting a more definite use of the procedure and in assessing results.

[1] Kagan, A. & Uemura, K. (1962) *Bull. Wld Hlth Org.*, 27, 667-679.

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## RÉSUMÉ

De 1960 à 1962, des essais effectués sous l'égide de l'OMS ont permis à un groupe de pathologistes de définir avec exactitude les différents types de lésions anatomo-pathologiques dues à l'athérosclérose au niveau de l'aorte et des artères coronaires, de choisir une méthode macroscopique standard pour leur classification et de préciser les techniques et les critères à employer.

Le présent travail rapporte les premiers résultats de l'application de cette méthode à l'examen de matériel anatomique prélevé au cours d'autopsies.

Après une étude préalable des statistiques de mortalité et d'autopsies, on a choisi sept groupes de population démographiquement définis où existait une probabilité de pouvoir effectuer l'examen nécropsique de 80 à 90% des personnes décédées. Dans six de ces communautés, depuis le début de 1963, on a procédé de façon uniforme à la récolte du matériel et à l'établissement des données statistiques qui s'y rapportent. Dans une des régions, 80-90% des décès de personnes âgées de 40 à 79 ans ont été inclus dans l'enquête. Pour l'ensemble des groupes, les autopsies suivies de prélèvement ont été effectuées dans 56,4-75,4% des décès, et pour l'un des groupes, on obtint même de pouvoir procéder à l'autopsie de 95% du nombre des personnes décédées à domicile.

Jusqu'à présent, du matériel destiné à l'enquête a été prélevé sur environ 6000 sujets. Les pièces anatomiques ont été préparées de façon identique, et 3000 d'entre elles ont été examinées au cours d'une série de 4 séances de classification des lésions d'athérosclérose de l'aorte et des artères coronaires.

Les auteurs décrivent en détail la technique adoptée.

Chaque séance est précédée d'une phase de préparation au cours de laquelle les examinateurs se mettent d'accord sur les définitions et les critères. Ils ne disposent d'aucune information sur l'origine de la pièce qu'ils examinent. Des échantillons de même origine sont répartis de façon égale entre tous les observateurs afin de neutraliser toute évaluation systématiquement divergente. Chaque séance quotidienne est divisée en quatre séances partielles, ce qui permet de soumettre à un nouvel examen des pièces déjà présentées. On parvient ainsi à déceler les divergences dans la cotation chez un même observateur, ainsi que les différences d'appréciation pour l'ensemble des examinateurs à l'occasion de deux séances partielles distinctes; il est dès lors possible d'étalonner la méthode.

Chez un même examinateur, les divergences n'excèdent pas en général 10%; elles s'observent principalement lorsqu'il s'agit d'évaluer l'importance des lésions compliquées de l'aorte et d'apprécier l'ordre de grandeur des dépôts lipidiques, des plaques fibreuses et des lésions compliquées des artères coronaires. Elles sont moins accentuées en revanche lorsque l'examen porte sur la « quantité totale d'athérosclérose » présente dans les préparations. Quant aux divergences d'ensemble, elles sont de l'ordre de 5%.

On a pu démontrer la valeur pratique de la méthode et évaluer son pouvoir de discrimination: une différence de moins de 15% décelée entre deux groupes de préparations correspond à une différence réelle ($P = 0,05$), même si l'examen ne porte que sur 50 échantillons de chaque groupe pour l'athérosclérose, et sur 100 pièces de chaque groupe pour la sténose coronaire. Ces résultats sont supérieurs aux prévisions.

Les auteurs présentent en annexe la liste des définitions adoptées pour la classification des lésions, la technique de préparation et de coloration des pièces anatomiques, et donnent le détail des renseignements de nature statistique qui sont rassemblés lors de chaque prélèvement.


*Annex 1*

## STANDARD OPERATING PROTOCOL FOR WHO COMBINED EPIDEMIOLOGICAL AND PATHOLOGICAL STUDIES OF ATHEROSCLEROSIS

A meeting on Combined Epidemiological and Pathological Studies of Atherosclerosis was held in Moscow from 19-24 March 1962, with the following participants: Dr A. Whitley Branwood (*Rapporteur*), Department of Pathology, University of Aberdeen, Aberdeen, Scotland; Dr Aubrey Kagan (*Secretary*), Medical Officer, Cardiovascular Diseases, World Health Organization, Geneva, Switzerland; Professor F. Linell, Chief, Department of Pathology, Allmänna sjukhuset, Malmö, Sweden; Dr C. Tejada, Chief, Department of Pathology, Instituto de Nutrición de Centro América y Panamá, Guatemala; Mr K. Uemura, Chief Statistician, Health Statistical Methodology, World Health Organization, Geneva, Switzerland; Professor R. Vaněček, Deputy Director, II Department of Pathology, Charles University, Prague, Czechoslovakia; Professor A. M. Vihert (*Chairman*), Chief, Department of Pathology, Institute of Therapy, Academy of Medical Sciences, Moscow, USSR.

Existing data and the results of previous WHO trials of methods of grading atherosclerosis [1] were considered, and the following Standard Operating Protocol [2] was drawn up for use in epidemiological studies.

## SOURCE OF MATERIAL

### *Epidemiological control*

The material should be obtained from deaths related to demographically defined groups or subgroups of people. To decide whether a study can be made of a community it is necessary to have data on the number of deaths occurring in that community by age, sex and cause and on the number of these deaths likely to be available for post-mortem examination. Additional information about those deaths from which post-mortem material cannot be obtained is required, e.g., death certificate information on cause of death, occupation. Adequate information of this sort also implies knowledge of which deaths from the community under study occur outside the community area and which deaths occurring within the community area are related to the community and which are not.

In some communities it is possible to study material from nearly all deaths. In others this is not possible but comparison may be made with material from all deaths occurring in a particular age-group or, less usefully, due to a particular cause, e.g., forensic, " ischaemic heart disease ", cerebral accident, hypertension, neoplasm.

### *Age limits*

There is little interest at the present time in material from subjects under the age of 10 years and this is the agreed lower limit. The limits should be determined by the interest presented by and the availability of epidemiologically representative material. In some communities material from people over the age of 70 years is plentiful and it is expected that a short period of study will produce sufficient data.

When this stage has been reached, material from older age-groups will not be required and efforts may then be turned to studying more material from younger age-groups. The surplus older material can then be used to demonstrate the relative value of different methods, e.g., transverse section of coronary arteries as against longitudinal section, or for other procedures.

## DEFINITIONS OF ANATOMICAL AND PATHOLOGICAL TERMS

*Aorta, abdominal*: The aorta from a horizontal line drawn through the upper edge of the orifice of the coeliac artery to a horizontal line drawn through the inner surface of the bifurcation of the aorta.

*Aorta, complete*: The aorta from just above the aortic valves to the bifurcation.

*Aorta, descending thoracic*: The aorta from a horizontal line drawn through the first two intercostal arteries to a horizontal line drawn through the upper edge of the orifice of the coeliac artery.

*Atherosclerosis*: A variable combination of change of the intima of arteries, consisting of the focal accumulation of lipids, complex carbohydrates, blood and blood products, fibrous tissue and calcium deposits, and associated with medial changes.

*Calcification*: Areas in which there is calcium deposition detectable either visually or by palpation *without* overlying haemorrhage, ulceration or thrombosis.

*Complicated lesions*: Those areas in which there is ulceration, haemorrhage or thrombosis with or without calcium deposits.

*Coronary, circumflex*: The circumflex branch of the left coronary artery from its origin, excluding auxiliary branches. (If there are two vessels apparently equal in size, both should be taken.)

*Coronary, left anterior descending*: The left coronary artery from its orifice (including the ostium) and the anterior descending branch down to the apex of the left ventricle, excluding any branches. (If there are two vessels apparently equal in size, both should be taken.)

*Coronary, right*: The right coronary artery from its origin (including the ostium) and including the flexure at the margin of the posterior interventricular septum, excluding any branches.

*Coronary stenosis*: Narrowing of the lumen of the coronary artery by more than 50%.

*Fatty streak*: Any intimal lesion that is stained distinctly by Sudan IV or other fat-soluble dye and

[1] Kagan, A. & Uemura, K. (1962) *Bull. Wld Hlth Org.*, **27**, 667-679.

[2] Revised in February 1963. This protocol was developed in close co-operation with the International Atherosclerosis Project (PIA) and efforts were made to keep it as similar as possible to the PIA protocol (see page 298).

that does not show any other type of change underlying it.

*Fibrous plaque*: Any firm, elevated intimal lesion which in the fresh state is pale grey, glistening and translucent. After staining it may be partially or completely covered by sudanophilic deposits. If a lesion presents any haemorrhage, thrombosis, ulceration or calcification, that portion will be classified under those categories and not as a fibrous plaque. (For operational purposes the term " atheroma " is included in this definition though it is realized that controversy exists about the word.)

## PREPARATION OF SPECIMENS

### *Dissection of aorta*

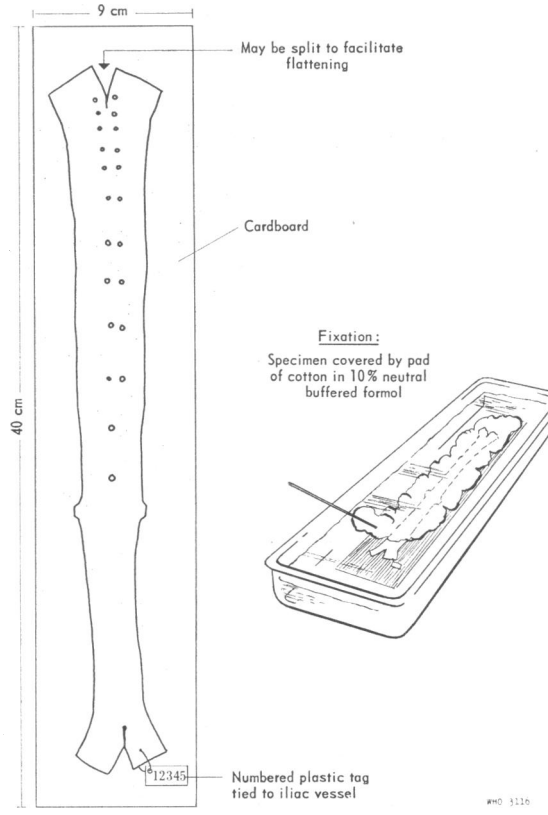
The aorta is removed from the ligamentum arteriosum to a point just beyond its bifurcation into the iliacs in one intact segment so far as possible. The arch and major branches are discarded.

The intercostal, coeliac, superior and inferior mesenteric and renal arteries are severed at their orifices, so that none of the branch remains on the adventitial surface to produce wrinkling or distortion of the aortic wall when it is fixed. The aorta may be opened either posteriorly or anteriorly, depending on whichever procedure fits the autopsy technique being used. Although the incision may be either anterior or posterior, it should be as straight as possible in order that the opened specimens have as near a rectangular outline as possible. If the specimen is to be kept for any prolonged period of time before final dissection and fixation it should be moistened with saline solution and kept in the refrigerator in order to avoid post-mortem haemolytic staining. After opening, the major portion of the adventitial fat and connective tissue is removed. The aorta is then labelled with a prenumbered (accession number) plastic tag tied through a perforation made in the thoracic aorta *above* the highest intercostal arteries or through the iliac below the bifurcation. It is imperative that this number correspond to the number of the basic information sheet filled out for the same case.

The aorta is placed on a piece of cardboard cut to a convenient size (approximately 9 × 40 cm) with the adventitia against the cardboard (Fig. 7). The cardboard must be dry and the aorta must not be dripping wet in order to ensure adherence of the specimen to the cardboard. The cardboard, together with aorta and label, are then placed under 10% neutralized formol in a flat tray or pan with a cover.

FIG. 7

PREPARATION OF AORTA

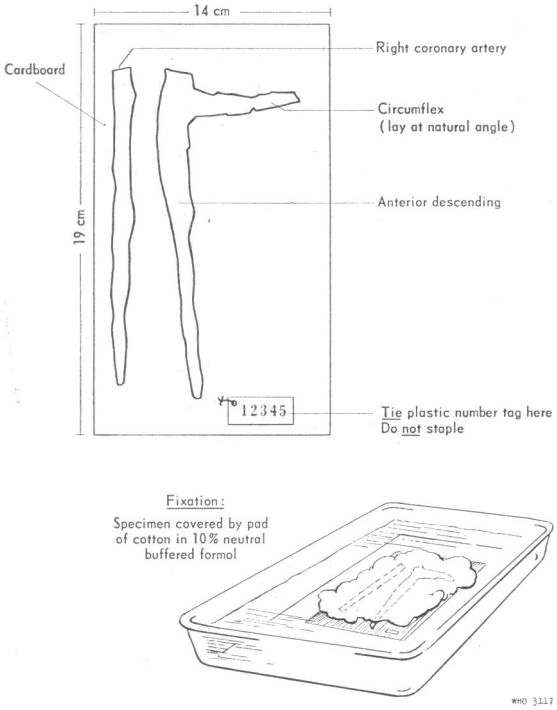



The aorta is covered with a thin layer of cotton (*not* gauze or towel), over which may be placed another specimen. Care should be taken at all steps that the identifying tag does *not* lie over or under the specimen, since this produces an indentation in the artery wall. After at least 24 hours of fixation, the aorta is removed from the formol and stripped from the cardboard. It is then placed in a plastic bag 15 × 45 cm together with the label ; approximately 50 ml of 10% formol are poured into the bag, which is then compressed between the foam-rubber liners of a specially constructed press. Excess formol is permitted to drain off and the top of the bag is heat-sealed. The aorta is then ready for transport to " Central Services ".[1]

[1] " Central Services " refers to the Department of Pathology, Allmänna sjukhuset, Malmö, Sweden.

FIG. 8

PREPARATION OF CORONARY ARTERY




*Coronary arteries*

Before the heart is opened the three main branches of the coronary arteries, including the ostia, are opened by slitting longitudinally with blunt-pointed iris scissors (see " Definitions ", above, for a description of the complete coronary segments). To avoid haemolytic staining of the intimal surface, wash the heart with physiological saline. The opening slit should be as straight and neat as possible. There are cases, however, when owing to calcification of the walls, it is not possible to open the vessel completely. As much of the vessel as possible proximal and distal to these parts should be opened. Coronary arteries are acceptable if the heart has been opened in such a manner that not more than one cut across any one main branch is made, such as the common method of opening the left ventricle produces when the circumflex branch of the left coronary artery is severed near its origin. Any coronary arteries that have been cut more than once prior to dissection must be excluded. The statistical branch of " Central Services " must be informed.

With general traction from the distal end, the coronary arteries are dissected from the heart with a minimum of epicardial fat attached. Once the coronary arteries have been separated from the heart, adventitial adipose tissue is removed by careful dissection. Removal of adventitial adipose tissue is important for proper evaluation of the lesion either visually or by machine. After removal of excess epicardial fat the coronaries are placed on cardboard approximately 14 × 19 cm, as shown in Fig. 8. They are labelled with the accession number by tying a pre-numbered plastic tag through a perforation in one corner of the cardboard. They are then fixed in formol in the same way as the aortas. After fixation they are placed in a plastic bag one half the length of that used for aortas. A small amount of formol is included in the bag as for the aorta, and it is then placed in the press and sealed.

*Centralizing and storing*

" Central Services " are provided to: (*a*) control the numbering of the specimens; (*b*) check suitability of material for grading and basic information; (*c*) offer comments to collaborators concerning the preparations, where necessary; (*d*) prepare the material for grading by the investigating pathologists; and (*e*) provide statistical evaluation and control.

When a suitable number of specimens has been collected they are sent to the Department of Pathology, Allmänna sjukhuset, Malmö (" Central Services "), and the relevant basic information forms to the Cardiovascular Diseases unit, WHO, Geneva. Close control of the movement of specimens is ensured by separate postage of a list of specimens to be graded and return of a receipted postcard after delivery. All stained specimens are stored at " Central Services ", and are then available for evaluation, other special studies, or return to the original laboratories.

*Staining*

In order to stain the specimens uniformly with Sudan IV they are placed in a specially designed apparatus. The staining solution, time and temperature are also standardized (see Annex 2). The specimens are cleaned and again resealed in plastic bags identified only by the accession number.

## GRADING OF LESIONS

Coronary arteries and aortas are graded by a team.

Specimens for grading are allocated to the examining pathologists by the statistician. This facilitates

the introduction of the necessary statistical checks. With the present grading methods it is necessary to allocate an equal proportion of specimens from each source to each examining pathologist.

*Grading sessions*

The examining pathologists are responsible for the visual method of evaluation, under the control of the statistician.

A set of standard specimens is prepared for ensuring comparison between the examining pathologists and this is exchanged from time to time with PIA or other workers.

*Visual estimation*

In the process of grading, the first step is to estimate the total percentage of the intimal surface involved with all types of atherosclerotic lesions in each of the areas to be considered (thoracic aorta, abdominal aorta, right coronary, left circumflex coronary and left anterior descending coronary). The percentage of the entire involved surface due to each of the four different types of atherosclerotic lesions (see " Definitions " above) is then estimated.

For coronary arteries an attempt is made to estimate the presence or absence of stenosis that is greater than 50% of the lumen. This is recorded for the right, left and circumflex coronary arteries independently, and the presence of one or more such areas of narrowing per artery is recorded. All these data are recorded on the forms shown in Fig. 9 and 10. One form, numbered appropriately, is provided for each observer and each specimen.

At no time during the grading is the pathologist aware of the source of the specimens or of any characteristic of the case from which the specimen was obtained.

The aorta is graded in two segments separately, the descending thoracic portion and the abdominal portion (see " Definitions " above).

FIG. 9

FORM FOR RECORDING VISUAL GRADING OF ATHEROSCLEROTIC LESIONS OF AORTA [a]

**WORLD HEALTH ORGANIZATION**

Combined Epidemiological and Pathological Studies of Atherosclerosis

Visual grading - Aorta

1-2. Study number: 30 3-7. Accession number:
8. Grading session: 9. Observer:

| Aorta | Total amount atherosclerosis | Fatty streak | Fibrous plaque | Complicated | Calcified | Diffuse redness (tick if present) |
|---|---|---|---|---|---|---|
| Descending thoracic | 10-11 | 12-13 | 14-15 | 16-17 | 18-19 | |
| Abdominal | 20-21 | 22-23 | 24-25 | 26-27 | 28-29 | |

WHO 41444

[a] Grader enters estimated percentage of aorta involved.

FIG. 10

FORM FOR RECORDING VISUAL GRADING OF ATHEROSCLEROTIC LESIONS OF CORONARY ARTERY [a]

**WORLD HEALTH ORGANIZATION**

Combined Epidemiological and Pathological Studies of Atherosclerosis

Visual grading - Coronaries

1-2. Study number: 30 3-7. Accession number:
30. Grading session: 31. Observer:

| Coronary | Coronary* stenosis > 50% | Total amount atherosclerosis | Fatty streak | Fibrous plaque | Complicated | Calcified | Diffuse redness (tick if present) |
|---|---|---|---|---|---|---|---|
| Right | 32 ☐ 1 None ☐ 2 1 Area ☐ 3 >1 Area ☐ 4 Unknown | 33-34 | 35-36 | 37-38 | 39-40 | 41-42 | |
| Left anterior descending | 43 ☐ 1 None ☐ 2 1 Area ☐ 3 >1 Area ☐ 4 Unknown | 44-45 | 46-47 | 48-49 | 50-51 | 52-53 | |
| Left circumflex | 54 ☐ 1 None ☐ 2 1 Area ☐ 3 >1 Area ☐ 4 Unknown | 55-56 | 57-58 | 59-60 | 61-62 | 63-64 | |

*Tick appropriate square

WHO 41445

[a] Grader enters estimated percentage of coronary artery involved.

*Observer variation*

Grading is under the supervision of the statistician. Tests of inter- and intra-observer variation and inter-session variation are carried out. In this way the comparability of different observers can be ascertained, the procedure can be adjusted to provide maximum efficiency, and the imprecision of the method can be estimated.

*Objective methods*

Such methods will be sought in collaboration with other investigators.

## BASIC INFORMATION

*Preparing and issuing forms*

The sets of forms to be used for recording data are prepared by WHO. Each set is identified by a five-digit serial number and accompanied by a set of two similarly numbered plastic tags. " Central Services " assign at random accession numbers to the participating laboratories. Records are kept of these assignments, and forms and tags are sent as completed material is received. Each participant is notified of any assigned to him, and a written acknowledgement of receipt is requested. Any losses or damages to the material forwarded to them and any errors or inconsistencies are to be notified to " Central Services ".

*Basic information form*

This form is shown in Fig. 11. Each aspect to be recorded is given a numbered heading and each category of this aspect is given a square for recording purposes. Large squares are intended for entering numerical data and small ones for ticking. In the latter case *one* square, and one square only, must always be ticked under *every* heading. The criteria are defined below.

FIG. 11

BASIC INFORMATION FORM USED IN WHO ATHEROSCLEROSIS STUDIES

**WORLD HEALTH ORGANIZATION**

COMBINED EPIDEMIOLOGICAL AND PATHOLOGICAL STUDIES OF ATHEROSCLEROSIS

3-7 Accession number

8 Name of hospital: Code

Date of death:

9-12 Autopsy number

13 Place of residence: Urban 1 / Rural 2 / Unknown 9

14 Co-operating pathologist: Code

15 Source: Accident 1 / Not accident: Hospital 2 / Home 3 / Unknown 9

16 Medical care before death: Yes 1 / No 2 / Not sure 9

17 Was death sudden? (Death occurring unexpectedly within 6 hours in an apparently healthy subject or in a sick person whose condition was either steady or improving): Yes 1 / No 2 / Unknown 9

18 Was diagnosis made before post-mortem? Yes 1 / No 2 / Unknown 9

19-21 If yes, clinical diagnosis: Code

22-23 Date of birth: day mo yr Code

24 Sex: Male 1 / Female 2

25 Ethnic group: Code

26-27 Occupation: Code

28-30 Weight kg

31-33 Stature (length) cm

34-36 Fat: panniculus between xiphoid and umbilicus mm

37-39 Heart weight after removing coronary arteries gr

40 Clinical hypertension: Benign essential hypertension 1 / Malignant hypertension 2 / Other hypertension 3 / Absent 4 / Unknown 9

41 Diabetes mellitus: Present 1 / Absent 2 / Unknown 9

42 Syphilis aorta (macroscopic): Present 1 / Absent 2 / Doubtful 3

Coronary occlusion:

43: Present 1 / Absent 2 / Uncertain 3

44: Due to thrombus 1 / Not due to thrombus 2 / Not applicable 3 / Not sure 9

45 Recent myocardial infarction: Single 1 / Multiple 2 / None 3

46 Scar due to myocardial infarction greater than or equal to 0.5 cm: Single 1 / Multiple 2 / None 3

47 Small scar less than 0.5 cm: Single 1 / Multiple 2 / None 3

48 Coronary occlusion without infarction as the cause of death: Present 1 / Absent 2 / Unknown 9

Cerebrovascular accident:

49 Post-mortem recent lesion: Haemorrhage 1 / Infarction 2 / One or other (not sure which) 3 / None found 4 / Not examined 9

50 Post-mortem old lesion; cyst greater than 0.5 cm: Present 1 / Absent 2 / Not examined 9

51 Disabling peripheral vascular disease (Intermittent claudication; Raynaud's syndrome; gangrene or amputation of extremity due to occlusive disease of arteries) (Underline appropriate categories): Present 1 / Absent 2 / Unknown 9

52 Other diseases due to atherosclerosis (Atherosclerotic aneurysm; carotid, mesenteric, coeliac, renal or other artery occlusion by atherosclerotic lesion) (Underline appropriate categories): Present 1 / Absent 2 / Not sought 9

53 Pathological confirmation of clinical diagnosis: Yes 1 / No 2 / No clinical diagnosis 3 / Not sure 9

54-59 Pathological diagnosis:

Signature:

WHO 41446

Basic information forms should also be completed as far as possible for deaths in the study but for which autopsy material is not available. These forms do not have an accession number and are labelled " Incomplete ".

*Accession number (entries 3-7).* A serial number is printed in this space by " Central Services ". This number and form will be sent in duplicate to the collecting laboratory. If a numbered sheet is damaged it is sent to " Central Services " marked " void "; if one or more sheets are lost, the missing numbers should be reported to " Central Services ".

*Date of death.* The date of the death of the subject.

*Name of hospital (entry 8).* This refers to the hospital at which post-mortem examination is made. Provision is made for a code number.

*Autopsy number (entries 9-12).* The hospital number for the autopsy.

*Place of residence (entry 13).* Record in the appropriate place whether urban, rural or unknown. Each laboratory will establish a policy concerning the definition of " urban " and " rural " for its local situation.

*Co-operating pathologist (entry 14).* The name of the pathologist performing the post-mortem and completing the basic data form. His signature should appear at the bottom of the form. Provision is made for a code number.

*Source (entry 15).* The object is to discriminate between violent death from any source and non-violent death. The latter is subclassified according to whether death occurs in hospital or elsewhere, e.g., home.

*Medical care before death (entry 16).* The appropriate square should be completed.

*Was death sudden ? (entry 17).* The object is to see how many deaths coming within a practical definition of " sudden " are due to coronary occlusion. Accidental death or death due to intentional trauma is excluded. The definition suggested is " death occurring unexpectedly within six hours in an apparently healthy subject or in a sick person whose condition was either steady or improving ".

*Pre-autopsy diagnosis (entries 18 and 19-21).* Where a definite pre-autopsy diagnosis is given or where a single condition is indicated, the square " Yes " is ticked and the diagnosis is recorded in entries 19-21. Where a diagnosis is made but several other possibilities are mentioned, square " Yes " is ticked in entry 18 and the diagnosis (not the possibilities) is recorded in entries 19-21 and coded centrally. Where several pre-autopsy diagnoses are given but all are given as doubtful, square " No " is ticked. Where there is insufficient evidence to come to a conclusion the square " Unknown " is ticked in entry 18.

*Date of birth (entries 22-23).* This refers to the date of birth of the subject and should be entered in the form of numerals for the date, followed by numerals for the month, followed by the last two numerals of the year—e.g. 21 August 1916 is entered as 21 08 16. When this date is not available, the next most accurate date is entered (e.g., -- 08 16) or, finally, the age in years.

*Sex (entry 24).* Sex should always be recorded.

*Ethnic group (entry 25).* The exact way in which this will be used has not been determined. For the time being it is mainly for use in the USSR. A decision on how to use it and what to code has not been made.

*Occupation (entries 26-27).* The last occupation should be entered descriptively. If any data relating to duration of this occupation and previous occupation are available, they should also be entered. Coding will be made centrally when a procedure has been devised.

*Weight (entries 28-30).* The weight of the whole body in kilograms, to the nearest kilogram, is entered when this is available—e.g., 0 7 5 kg. If these data are not available, a dash should be placed in each square (— — —).

*Stature (entries 31-33).* The length, in centimetres to the nearest centimetre, is recorded in the squares provided.

*Fat (entries 34-36).* The depth of subcutaneous fat, halfway between the xiphoid sternum and the umbilicus in the mid line, is measured to the nearest millimetre and recorded in the squares provided.

*Weight of heart (entries 37-39).* The heart is weighed (in grams) after removing the coronary arteries and opening the ventricles and auricles to remove blood and blood clots. The auricles and epicardial fat remaining after removal of the coro-

naries are included in the heart weight. The weight is recorded in three digits. If the heart is not weighed, a dash is placed in each square (— — —).

*Clinical hypertension (entry 40).* " Benign essential hypertension " means that a clinical diagnosis of hypertensive cardiovascular disease was made and that no chronic renal disease other than benign arteriolar nephrosclerosis was diagnosed either clinically or anatomically. This diagnosis is not based on the heart weight at autopsy. " Malignant hypertension " indicates a clinical diagnosis of malignant hypertension. " Other hypertension " indicates a diagnosis of hypertension secondary to another condition such as chronic renal disease, pyelonephritis, glomerulonephritis, eclampsia, pheochromocytoma, etc., as established at autopsy. " Absent " indicates that clinical diagnosis of hypertension was excluded. " Unknown " is to be recorded when there is no clinical knowledge of the presence or absence of hypertension clinically, as in the case of a patient arriving comatose at the hospital and dying with no medical history available, or accidental deaths autopsied at medico-legal laboratories.

In the absence of a diagnosis but the presence of blood pressure records, a record of pressure of 160/95 or more in subjects under 60 years of age or of 140/90 or more in subjects under 40 years of age would be regarded as indicating the presence of essential hypertension. In the absence of a diagnosis and the presence of records showing blood pressure less than these (except for pressures taken in shock or *in extremis*), hypertension would be regarded as absent. In other cases and in subjects aged 60 years or over, the square " Unknown " should be ticked. For subjects 60 years or over for whom a pressure has been recorded, that pressure should be shown on the form; if more than one record is available, the range should be given.

*Diabetes mellitus (entry 41).* Diabetes mellitus is reported as " absent " when a reasonably adequate medical examination has been performed (urine or blood examined for sugar) and no clinical diagnosis was made. " Unknown " is recorded when no clinical history or laboratory data of diabetes are available and the presence of diabetes cannot be reasonably excluded. " Present " is recorded when the information available makes the presence of diabetes clear.

*Syphilis aorta (entry 42).* This is recorded as " present " if there is *gross* evidence of syphilitic aortitis in any portion of the aorta (with or without aneurysm), insufficiency of the aortic valve due to dilatation of the aortic ring, or intimal plaques due to syphilitic aortitis obstructing the orifices of the coronary arteries. The diagnosis is made on the basis of gross appearance and not on histological study of the aorta. Record as " present ", " absent ", or " doubtful ".

*Coronary occlusion (entries 43 and 44).* The presence of coronary occlusion is to be recorded for the three coronary arteries as a whole. A record is to be made under one of the headings " present ", " absent ", or " uncertain " (entry 43); and under one of the headings " due to thrombus ", " not due to thrombus ", " not applicable ", or " not sure " (entry 44). In cases where there is more than one occlusion, some due to thrombus, others not, both squares should be ticked (the form was corrected later).

*Myocardial infarct (entries 45, 46 and 47).* The co-operating pathologist indicates his opinion in the appropriate squares. One square in each of entries 45-47 should be ticked in all cases. In the rare event that a large scar is present ($\geqslant$ 0.5 cm) that is *not* due to myocardial infarction, entry 46.3 is ticked and a note is made under " Pathological diagnosis " (entries 54-59).

*Coronary occlusion without infarction as cause of death (entry 48).* " Present " is recorded in cases of sudden or rapid death in which the principal cause of death is judged by the pathologist to be myocardial ischaemia due to coronary stenosis or occlusion (which in turn may be due to thrombosis, haemorrhage into a plaque, or growth of a plaque), but in which no myocardial necrosis is grossly detectable. In such cases death may have occurred within seconds after onset of symptoms, or as long as several hours after onset of typical signs of myocardial infarction. This entity may, furthermore, be considered " present " in cases with myocardial scars but no congestive failure as a result of the previous infarction. In such cases it may be presumed that a second occlusive episode developed that resulted in sudden or rapid death. Cases in which the occlusion is clearly due to embolism (e.g., bacterial endocarditis) should be excluded from this category. Tick the square " absent " for these cases and list the diagnosis in the final diagnosis. Record as " present ", " absent ", or " unknown ".

*Cerebrovascular accident (entries 49 and 50).* The co-operating pathologist indicates his macroscopic post-mortem findings in relation to a recent lesion (entry 49) and an old lesion (entry 50). " Cyst " should only be recorded as present if greater than 0.5 cm in diameter (entry 50).

*Disabling peripheral vascular disease (entry 51).* Record as " present " in any case in which there is a history of intermittent claudication, Raynaud's syndrome, gangrene or amputation of an extremity due to occlusive disease of the arteries of the extremities. Do not record as " present " if the condition is known to be due to trauma or embolism not related to atherosclerosis.

*Other diseases due to atherosclerosis (entry 52).* Record as " present " atherosclerotic aneurysm with or without rupture or occlusion of carotid, mesenteric, coeliac, renal or other arteries by atherosclerotic lesions. Do not include calcified aortic stenosis, calcified mitral ring, medial necrosis of aorta with or without dissecting aneuryms, Monckeberg's sclerosis or congenital aneurysm of the circle of Willis.

*Pathological confirmation of clinical diagnosis (entry 53).* The object is to confirm or refute a pre-autopsy diagnosis, if made (entries 19-21). If the post-mortem findings confirm the pre-autopsy diagnosis, the square " Yes " is ticked; if they refute it, square " No " is ticked. If there is no pre-autopsy diagnosis or no post-portem diagnosis, square " No clinical diagnosis " is ticked.

*Pathological diagnosis (entries 54-59).* Ample space is provided to enter all the pathological findings. Coding is made by " Central Services " according to an agreed procedure.

*Signature.* The completed schedule should be signed by the pathologist performing the post-mortem examination.

FIG. 12
STAINLESS-STEEL TANK WITH AGITATOR

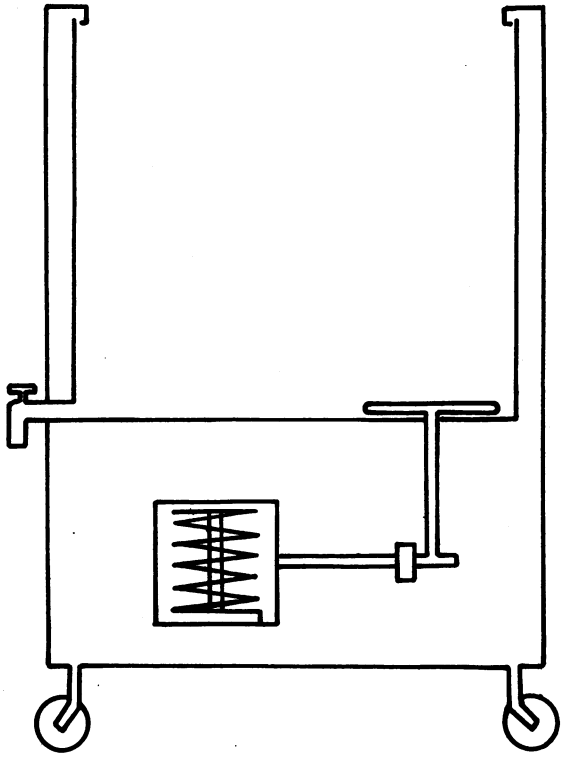
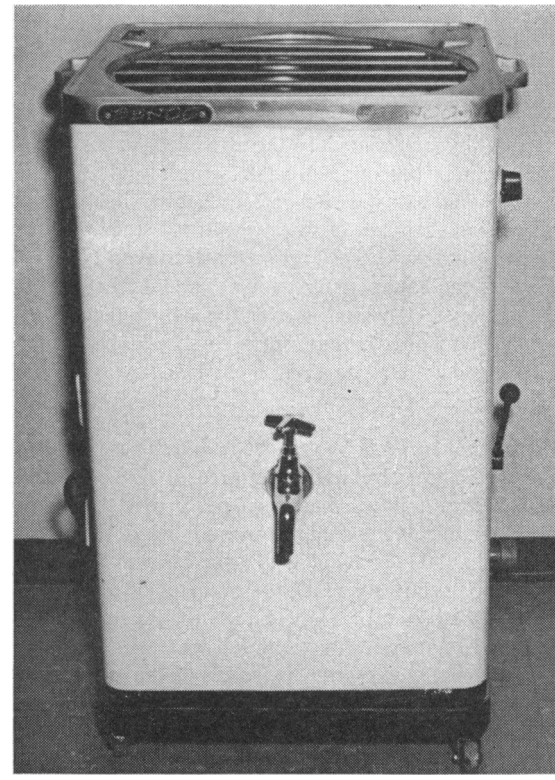


FIG. 13
VIEW OF STAINLESS-STEEL TANK, WITH LID OFF, SHOWING SUSPENSION BARS FOR AORTAS



*Annex 2*

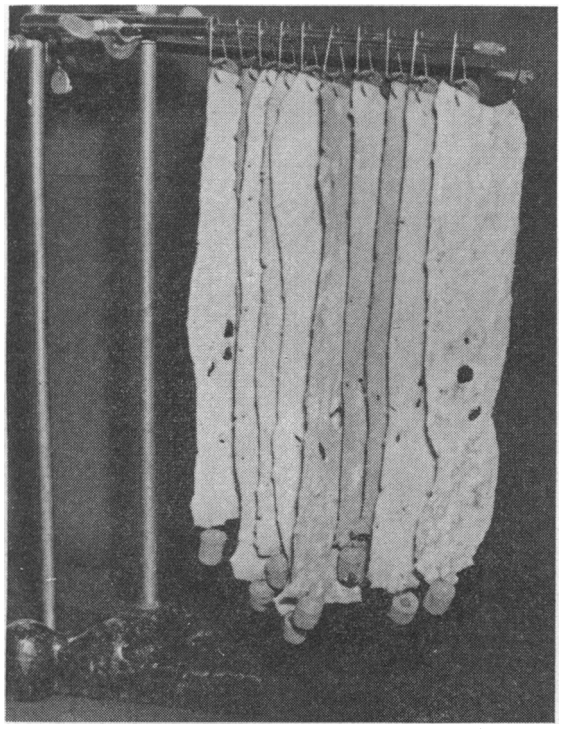
## STANDARD STAINING TECHNIQUE

A stainless-steel washing-machine has been adapted for this purpose. It holds about 60 litres of fluid. In order to get as even and constant staining as possible the fluid is kept in slow motion by the help of the original fan (rotator) at the bottom of the tank (Fig. 12 and 13). The egress is covered with a Petri dish to reduce the movement of the fluid.

The staining fluid is prepared from isopropyl alcohol (99% pure) and distilled water, to an alcohol concentration of 38%. The concentration is checked with a pyknometer. Supersaturated solution of Sudan IV in concentrated isopropyl alcohol is added until the dye concentration, checked with a colorimeter, gives an OD $\frac{524\ m\mu}{20°} = 0.23\text{-}0.24$. The staining solution has a pH of about 6.7. Measurements are made before each staining procedure and alcohol and saturated staining solution added until the correct concentration is reached. In spite of this the staining colour varies if the solution is used on many specimens. It is therefore completely renewed after each batch of specimens from 200-250 subjects.

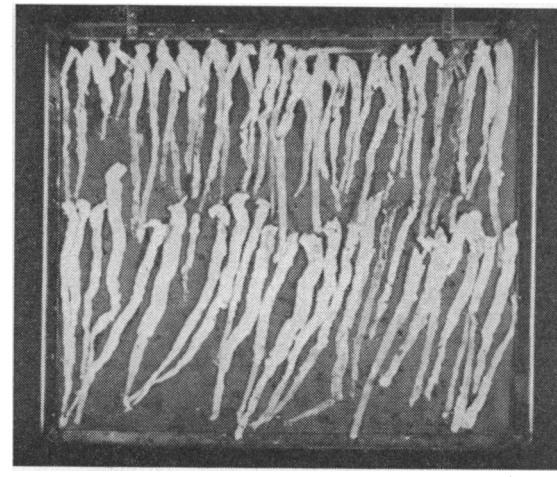
The machine holds either 70 aortas or 20 aortas plus 60 sets of coronaries. The aortas are suspended by numbered hooks of stainless steel (Fig. 14) and a note of the accession number corresponding to the hook number is made. To prevent the aortas from sticking together during the staining procedure a small lead weight is hung at the distal end of each. The hooks are suspended from the bars across the top of the tank (shown in Fig. 13). The coronary arteries are pinned to a stainless-steel net as shown in Fig. 15. The pins pierce each vessel at two points

FIG. 14
METHOD OF SUSPENDING AORTAS FROM SUSPENSION BARS [a]



[a] The numbered tag for identification is at the top of each aorta. A weight is attached to the lower end to keep the specimen straight and steady.

FIG. 15
METHOD OF PINNING CORONARY ARTERIES TO STAINLESS-WIRE MESH SUSPENDED IN THE STAINING TANK [a]



[a] Pins go through the specimens and wire mesh. Each specimen is numbered.

and are then stuck into small corks on the other side of the net. The specimens are placed in numerical order and the location is indicated on a special list. In this way a specimen can be identified even if one of the pins is lost or one of the arteries becomes completely detached. (This has not yet happened.)

The specimens are stained for 16-18 hours (overnight), during which time continuous agitation of the fluid is maintained and the tank is covered with an airtight lid.

After staining, the vessels are rinsed in running tap water for 1-3 hours. Before re-bagging they are cleaned from excess adventitial fat. The latter is very important for the grading procedure.

Examples of a stained aorta and a stained coronary artery are shown in Plates 1 and 2.

PLATE 1
STAINED AORTA

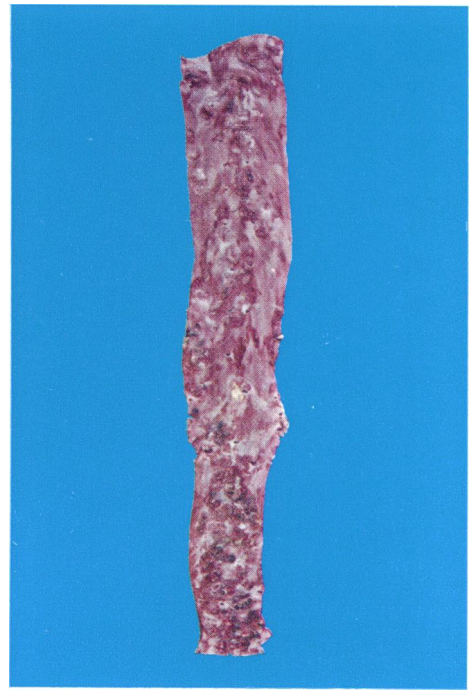


PLATE 2
STAINED CORONARY ARTERY