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RELATION OF SEVERITY OF ATHEROSCLEROSIS TO CHEMICAL COMPOSITION OF HUMAN AORTA

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Although the assessment of atherosclerosis in postmortem specimens is usually made by visual inspection of the intimal surface of arteries, selective staining with fat dyes, such as Sudan IV, has been used as a general procedure for the evaluation of atherosclerotic lesions. This method, however, can be criticized on the basis that fat dyes stain certain lipid material on the surface of the intima, and that cholesterol, fibrotic, and calcified tissue do not show sudanophilic properties. It has been shown, however, that the content of cholesterol in the aorta correlates very well with the degree of sudanophilia.[9]

The method recommended by the World Health Organization (WHO) study group on atherosclerosis[13] for the evaluation of atherosclerosis of the intima combines the proportion of areas showing sudanophilia with those with fibrotic and calcified lesions. This method gives a more quantitative assessment of the atherosclerotic involvement and has been used in many postmortem studies in different population groups. The WHO grading method has been used by the International Atherosclerosis Project.* There has been no attempt to correlate this grading with the chemical composition of the aorta.

In the present study, therefore, the chemical composition of the aorta was studied in relation to the degree of atherosclerosis obtained by Sudan staining as recommended by the WHO grading method,[13] using material collected in New Orleans and Guatemala.

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MATERIAL AND METHODS

A group of 49 aortas from individuals aged 20 to 80 years was selected at random from autopsy material collected at the Charity Hospital in New Orleans by the Department of Pathology of the Louisiana State University Medical School. Another group of 69 specimens of the same age range was selected in the same manner from the material collected at the General Hospital in Guatemala City by the Division of Pathology of the Institute of Nutrition of Central America and Panama (INCAP).

The vessels were opened anteriorly, stripped of adventitial fat, flattened on chipboard and fixed for 24 hr. in 10 % neutral formalin. The fixed specimens were placed in polyethylene bags containing a few milliliters of neutral formalin, and the bags were sealed by heat and shipped to the central laboratories at INCAP.

All of the specimens were stained with Sudan IV according to the WHO method.[13] After staining, the specimens were again placed in polyethylene bags, sealed, and saved for further grading and analysis.

Grading of Atherosclerosis

The grading of atherosclerosis was done independently by two observers, using the method recommended by the WHO study group on atherosclerosis,[13] which consisted of the following:

(a) Evaluation of the percentage of total area of intimal surface of aorta involved by atherosclerotic lesions.

(b) Evaluation of the percentage of aortic intimal surface involved by each of the following three main types of athero-

sclerosis: lipid streaks (sudanophilic), fibrous pearly plaques, and complicated lesions (hemorrhagic, thrombotic, ulcerated, and calcified).

In the evaluation of atherosclerosis the percentage of total involvement of the intima was obtained by visual observation and subjective estimation of the surface. This was recorded as the total percentage of involvement. The distribution of the different types of lesions was estimated as described above and given as a percentage of the area involved by atherosclerosis. A numerical profile for each specimen is ob-obtained in which the percentage of total involvement is given first and then the percentage of each of the lesions is considered. For example, a profile of 50: 100, 0, 0, for one particular specimen means that 50% of the intimal surface showed atherosclerotic lesions, but only lipid streaks were present. Another profile of 50: 50, 25, 25, means that 50% of the intimal surface was covered by atherosclerotic lesions but 50% of this area was lipid streaks, 25% was fibrous plaques, and 25% was complicated lesions. The percentage of the intimal surface area covered by any specific type of lesion is obtained by multiplying the percentage of area affected by atherosclerosis by the percentage of the lesion within the area affected. For example, in the first case given above, the lipid streaks were 50% of the total area of the aorta (50 × 1.00), whereas in the second case the lipid streaks were 25% (50 × 0.50), fibrous plaques were 12.5% (50 × 0.25), and complicated lesions were 12.5% (50 × 0.25).

Although these observations are subjective, the agreement between observers was usually very good. Sessions of standardization, however, were periodically conducted, and discrepancies in evaluation were properly corrected.

This grading, therefore, gives the percentage of area of the intima involved with atherosclerosis, the proportion of the individual lesions within the total area affected, and, by calculation, the percentage of area involved by individual lesions in relation to the total aorta.

Chemical Analysis

For the chemical analysis the aortas were taken out of the bags, and the fat visualized on the adventitial side by the Sudan staining was removed. The aortas were placed flat on drying pans and were dried in a lyophilizer machine. The dried specimens were weighed and then broken down into small pieces and ground in a laboratory-type Wiley mill. The material obtained was further pulverized and homogenized in a porcelain mortar. The samples were dried again and kept in closed containers for the chemical analysis. Fat, ash, calcium, phosphorus, and cholesterol content were determined. All methods used, with the exception of that for cholesterol, were those given by the AOAC.[3] Fat content was determined by continuous ether extraction in a micro-Soxhlet apparatus. The ash content was determined by ashing in a muffle furnace at 550 to 600 C. An ash solution suitable for the determination of calcium and phosphorus was prepared by dissolving in hydrochloric acid the ash obtained by calcination. Calcium was determined by permanganate titration of calcium oxalate[3] and phosphorus was determined by a modification of the Fiske and SubbaRow method.[8] Cholesterol was determined by the method of Abell and associates,[1] using an alcoholic potassium hydroxide autoclave hydrolysate.

Correlation Analysis

The samples were divided into three groups according to the specific types of lesions: (a) lipid streaks only, (b) fibrous plaques (with and without lipid streaks), and (c) complicated lesions (with and without lipid streaks, fibrous plaques or both). Within each group the aortas were ranked according to the involvement of the group-specific lesion. For instance, the "lipid streaks" group was ranked on the percentage of involvement of sudanophilia; on the other hand, the "fibrous plaque" group was ranked according to this lesion, regardless of the extent of the involvement of lipid streaks present. For the "complicated lesions" group, the ranking was made in the

TABLE 1

COMPOSITION OF AORTAS ACCORDING TO ATHEROSCLEROTIC LESIONS*

Component	Lipid Streaks (40)		Fibrous Plaques (30)		Complicated Lesions (48)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Fat	2.49	1.05	4.40†	1.90	10.68†	4.67
Cholesterol	1.25	0.74	2.40†	0.99	6.56†	3.17
Ash	0.71	0.68	1.51†	1.26	7.85†	6.47
Calcium	0.23	0.21	0.60‡	0.83	2.60†	2.30
Phosphorus	0.15	0.11	0.43	0.85	1.53†	1.36

* Composition given in Gm. per 100 Gm. of dry tissue. Numbers in parentheses are the numbers of cases; S.D., standard deviation.

† Significant at the 1% level of probability.

‡ Significant at the 5% level of probability, in the comparison of lipid streaks with fibrous plaques, and fibrous plaques with complicated lesions groups.

same way, regardless of the presence of lipid streaks or fibrous plaques. In addition, each group was also ranked according to the total involvement with all three types of lesions included. The content of each chemical component determined was ranked in each group, and Spearman's rank correlation coefficients with the aorta ranks were calculated.[11] Correlation coefficients were also calculated between chemical components.[11]

RESULTS

The chemical composition by individual atherosclerotic lesions is presented in Table 1. As expected, the more complicated lesions showed higher values. These differences were highly significant, with the exception of phosphorus concentration, in the comparison between the lipid streak and fibrous plaque groups.

Table 2 gives the Spearman's rank correlation coefficients between the ranks of individual specific lesion involvement and composition ranks. This table also gives the rank correlation coefficients between total atherosclerotic involvement within specific lesion groups and the ranking of the concentration of the different components studied.

When the individual involvement of the specific lesions is considered, all correlations were significant with the exception of lipid streaks with fat and with ash concentration, and of fibrous plaques with ash.

The severity of atherosclerosis, as given by the total involvement of the aorta, within specific lesion groups shows the same results as when individual lesion involvement was considered.

Highly significant rank correlations were also obtained when all aortas, regardless of the type of lesion, were ranked according to the total involvement of atherosclerotic lesions.

The product-moment correlation coefficients (r) between chemical components, according to the specific type of lesion, and considering all the specimens studied, are given in Table 3. All correlation coefficients were highly significant with the exception of ash *versus* fat for the lipid streak and fibrous plaque groups, and ash *versus* cholesterol for the fibrous plaque group.

Product-moment correlation coefficients between dry weight of aorta and the degree of involvement were calculated only for the Guatemalan group data because weights in the New Orleans group were not recorded. The results were as follows: lipid streaks, -0.092; fibrous plaques, 0.077; and complicated lesions, 0.646; with 27, 19, and 17 degrees of freedom (df), respectively. Only the correlation coefficient of complicated lesions with dry weight is statistically significant. If all specimens are considered, however, the correlation coefficient is also significant ($r = 0.629$, df 67).

TABLE 2

RANK CORRELATION COEFFICIENTS BETWEEN CHEMICAL COMPOSITION AND AORTIC INVOLVEMENT

Lesion	*df*	Fat	Cholesterol	Ash
Correlation with involvement of specific lesion				
Lipid streaks	38	0.298	0.617*	−0.105
Fibrous plaques	28	0.364†	0.504*	−0.050
Complicated lesions	46	0.622*	0.587*	0.611*
Correlation with total involvement				
Lipid streaks	38	0.298	0.617*	−0.105
Fibrous plaques	28	0.605*	0.553*	−0.118
Complicated lesions	46	0.654*	0.691*	0.470*
All specimens	116	0.811*	0.838*	0.618*

* Significant at the 1% level of probability.
† Significant at the 5% level of probability.

TABLE 3

CORRELATION BETWEEN CHEMICAL COMPONENTS ACCORDING TO ATHEROSCLEROTIC LESIONS

Variables	Lipid Streaks (38)*	Fibrous Plaques (28)	Complicated Lesions (46)	All Specimens (116)
Ash *vs.* fat	0.228	−0.009	0.381†	0.654†
Ash *vs.* cholesterol	0.652†	0.116	0.378†	0.660†
Fat *vs.* cholesterol	0.508†	0.721†	0.859†	0.929†
Ash *vs.* calcium	0.864†	0.984†	0.978†	0.986†
Calcium *vs.* phosphorus	0.919†	0.965†	0.689†	0.804†

* Numbers in parentheses represent degrees of freedom.
† Significant at the 1% level of probability.

DISCUSSION

Other investigators[2, 4-6, 10, 12] have performed chemical analyses including cholesterol determinations on lesions of atherosclerosis or on total aortas. Geer and colleagues[9] have correlated intimal sudanophilia with cholesterol concentration, but this was done only on different sections of a single specimen. Selective staining with Sudan IV has been recommended for a better visualization of atherosclerotic lesions, and the combination of this procedure with a direct observation of the intima for the detection of more complicated lesions has been a general method used in the evaluation of atherosclerosis.

The present study can be criticized on the basis that the chemical determinations were performed on samples already fixed and stained, assuming that this process removes part of the components analyzed. Geer and associates,[9] however, using the same procedure, found that only 5.6% of the total cholesterol content is removed during the entire process. This amount is rather small and probably came primarily from the surfaces of the vessel in closest contact with the solvents. No information is available on the removal of other components, but the values found in the present study do not differ greatly from those reported in previous determinations in the aorta.[2, 4-6, 10, 12]

Despite the effect of the staining process, the main objective in the present series was to evaluate how the severity of atherosclerosis, as estimated on stained specimens, reflects the levels of chemical components of the aorta. These components are those that accumulate during the development of atherosclerosis.

When the material is divided according to the three main types of lesion (*i.e.*, lipid

streaks only, fibrous plaques with and without lipid streaks and complicated lesions with and without other lesions) as presented in Table 1, highly significant differences were obtained between the groups, regardless of the area involved. This mainly confirms the severity ranking given to those lesions: *i.e.*, lipid streaks as the least severe, and complicated lesions as the most severe.

When the percentage of involvement of the specific lesions is taken into consideration, as given in Table 2, significant correlations with the chemical composition are obtained in most of the cases. The lack of correlation between lipid streaks and ash, and fibrous plaques and ash content, is understandable, assuming that the deposition of minerals is a process that starts later in the sequential events of the development of atherosclerosis. The lack of correlation between lipid streak involvement and fat content was unexpected, however, because lipid streaks are estimated exclusively by the sudanophilia of the intima. Because the whole aorta was stained, and assuming an undisturbed intimal surface, there is the possibility that Sudan stained only sudanophilic lipids in the outermost layer of the aorta. This, of course, does not explain the correlation obtained between intimal sudanophilia and cholesterol concentration because cholesterol, excluding low melting point esters, is not stained by this dye.[7] Geer and colleagues,[9] however, have shown a similar correlation and have explained this association by indicating that cholesterol, when present in increased amounts, is rather constantly associated with other lipids, such as triglycerides, which do stain with Sudan. Even if lipid material, especially triglyceride, is removed by the staining process, the Sudan uptake should be proportional to the sudanophilic material remaining in the tissue. Another possibility is that the evaluation by sudanophilia does not discriminate between deeply stained areas and those lightly stained, and that the same weight in the evaluation is given to all of them, regardless of the degree of staining. Furthermore it has been shown that Sudan dyes do not stain solid lipid material but color them intensely on melting.[7] Therefore, depending on the fatty acid composition, the fats in the specimens studied could escape staining at the temperature at which the procedure was applied.

Because of the high correlations obtained when the total involvement of the aorta is considered, it is concluded that simple visual evaluation of atherosclerosis gives as good results as do systems utilizing specific staining procedures. In addition, dry weight also correlates very well with atherosclerotic involvement. Therefore, the dry weight of the aorta is also a good index of the severity of atherosclerosis.

SUMMARY

The relation of the chemical composition of the aorta, with atherosclerosis evaluated by selective staining and visual estimation of lesions, is presented. In Guatemala 69 cases, with a range in ages of 20 to 80 years, were collected, and in New Orleans 49 cases of the same age range were obtained.

The specimens were fixed in formalin and stained with Sudan IV as recommended by the WHO study group in atherosclerosis. Total content of fat, cholesterol, ash, calcium, and phosphorus were determined. Rank correlation coefficients showed a good association between chemical components and individual involvement of atherosclerotic lesions. In addition, the total involvement of the aorta, regardless of the type of lesion, showed highly significant correlations with chemical components. Therefore, the simple visual evaluation of the areas covered by atherosclerotic lesions is as good an indicator of the accumulation of chemical components as are systems utilizing selective staining procedures.

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