CALCIUM, COLLAGEN, ELASTIN AND HEXOSAMINE LEVELS IN THE ARTERIES OF WHITES AND BANTU


The components of connective tissue not only form the architectural units of the arterial wall, but they participate actively in the vital dynamic function of the blood vascular system. Like most other tissues, connective tissue is affected by age, and changes such as fragmentation and calcification in the elastic tissue have been reported. In the arteries these changes are apparently closely related to atherosclerosis.1,2

At present it is fairly well documented that atherosclerosis develops at an earlier age and progresses more rapidly in the aorta and coronary arteries of White than of non-White South Africans.3 This racial difference apparently does not exist in the cerebral arteries.4,5

The coronary and cerebral arteries are exposed to the same arterial blood and therefore to the same intra-arterial influences. Differences in vascular composition might therefore be of more than academic interest in regard to the aetiology of atherosclerosis. In an effort to throw further light on this question, a quantitative chemical analysis of the calcium, collagen, elastin and hexosamine content of the aorta, coronary and cerebral arteries of White and Bantu subjects was undertaken and is here reported.

MATERIAL AND METHODS

The arteries were obtained of consecutive autopsies at the Pretoria General Hospital. (Syphilitic and tuberculous cases were excluded.) The present report includes data for males of 10-19 years and 30-69 years, and females of 30-59 years. The blood-vessels were removed as previously described by Meyer et al.6 After removal of the vessels they were incised and, in the case of the aorta and coronary arteries, the adventitial layer was stripped. The atherosclerotic index for each vessel was assessed according to the method of Gore and Tejada.7

After the degree of atherosclerosis of the vessels had been assessed, the aortas were cut up into small portions about 6 cm. thick and dried to constant weight for 24-36 hours in a vacuum desiccator over concentrated sulphuric acid. This procedure removed about 95% of the moisture. The vacuum-dried material was ground in a Wiley mill fitted with a 20-mesh sieve, and if not analysed immediately, was stored at -2°C in sealed glass containers. The coronary and cerebral arteries were dried in like manner except that in the majority of cases it was unnecessary to cut the vessels up into small pieces.

After 0.1-0.2 G of the dry material of each vessel had been individually ashed at 600°C, the calcium content of the ash was determined by a micro-cretic-method based on Vogel's macro-method.8 The hexosamine, collagen and elastin levels of the different vessels were determined on 0.08-0.1 G of the fat-free material. Hexosamine was determined colorimetrically by Elson and Morgan's modification9 of the procedure described by Boas10 and by Kirk and Derby.11 The fat-free material was hydrolysed and the collagen and elastin content estimated from the hydroxyproline content of the hydrolysates. Hydroxyproline was determined according to the procedure described by Neuman and Logan12 as modified by Martin and Axelrod,13 and then converted to its equivalent of collagen or elastin by multiplying by the factor 7.46 (collagen) or 52.3 (elastin).

Hexosamine, collagen and elastin as percentages of the fat-free material were converted to percentages of the absolute dry material by multiplying by the total weight of fat-free material and dividing by the total weight of the absolute dry material in each case.

Total lipids as a percentage of the total absolute dry weight of each blood-vessel were determined according to the method of Böttcher.14

RESULTS

The mean values of the various constituents studied in the aorta, coronary and cerebral arteries are presented in Table I. Age, atherosclerotic index and the levels of calcium, collagen, elastin, hexosamine and total lipid were correlated with each other. This was done with the IBM 1620 digital computer of the University of Pretoria.

Since the correlation coefficient (r) does not take the effect of sample size into account, it was decided to test whether the correlations between the various vessel constituents differed significantly from zero by calculating the transformed standardized values (t) of the correlation coefficients according to the method described by Kenney and Keeping.15 A value of t greater than 2.33 or smaller than -2.33 was considered significant. A 2% level of significance (2.33) was chosen because of the great number of comparisons between dependent quantities that had to be made.

The normalized values (t') of the test statistic testing for differences between the mean values of the different vessel constituents in the different age and sex groups of the two races were analysed by the method described by Kenney and Keeping.16 As in the first method, a t-value greater than 2.33 or smaller than -2.33 was considered significant. A positive value for t' indicated a greater mean value for White subjects than for Bantu subjects and a negative value a greater mean value for Bantu subjects.

ATHEROSCLEROTIC INDICES

The data on the degree of atherosclerosis in the 3 vessels of the 2 races, as estimated macroscopically by means of the atherosclerotic index method of Gore and Tejada,17 have been reported on by Meyer et al.9

Calciurn

Age. The mean calcium concentration of the 3 types of blood-vessels increased with increasing age in both sexes of the 2 races (Table I). Except in the case of the cerebral arteries of White subjects the correlation between calcium and age was statistically significant.

Atherosclerotic index. In general significant positive correlations were obtained between calcium concentration and atherosclerotic index.

Vascular beds. Calcium increased less rapidly in the cerebral arteries than in the aortas and coronary arteries of White subjects. It constituted about the same percentage of the total dry weight of the aortas as of the coronary arteries of White males, but a much smaller proportion of the dry weight of the cerebral arteries. In White females and in non-Whites of both sexes calcium constituted a much higher percentage of the total dry weight of the aortas than of the coronary and cerebral arteries (Table I).

Up to the age of 50 years calcium constituted a higher percentage of the mean dry weight of the cerebral arteries of Bantu than of their coronary arteries (Table I). In general the calcium level of the aorta showed a significant positive correlation with that of the coronary arteries, but in the case of the aortas and cerebral arteries a positive correlation was shown only by Bantu. There was no significant correlation between the calcium concentration of the coronary arteries and that of the cerebral arteries.

Race. Up to the age of 40 years the calcium concentration of the aortas in the 2 races showed only slight differences.

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Thereafter calcium increased much more rapidly in Whites than in Bantu. In the coronary arteries racial differences were already present in the 30-39 years age group (Table I). Beyond the age of 40 years the mean calcium concentrations of aortas and coronary arteries of White males were higher than the corresponding values in Bantu males, the differences being statistically significant. Significant race-related differences in the calcium concentrations of the cerebral arteries were not noticed.

Collagen

Age. The collagen concentration of aortas in both races and coronary and cerebral arteries in Whites decreased with increasing age (Table I). In the aortas of males this negative correlation with age was statistically significant.

_Atherosclerotic index_. Collagen decreased as the atherosclerotic index increased. The negative correlations between these 2 characteristics were greater in the aortas and coronary arteries of White subjects than in the corresponding arteries of Bantu (Table I).

_Other constituents_. Negative correlations were observed between collagen and calcium and between collagen and total lipids. The correlations were again greater in the aortas and coronary arteries of White males than in the corresponding arteries of Bantu (Table I).

_Vascular beds_. Of the 3 different vascular beds, the aortas showed the lowest and the cerebral arteries the highest mean collagen concentration (Table I). Significant positive correlations between the collagen concentrations of coronary and cerebral arteries were found in White females and in Bantu males.

_Race_. The tendency for collagen to decrease with increasing age was somewhat more pronounced in the aorta and coronary arteries of White males than in the corresponding arteries of Bantu males. In the coronary arteries this racial difference was significant (Table I).

_Elastin_. Age. In general the mean elastin concentrations of aortas, coronary and cerebral arteries decreased in both races with increasing age (Table I). These changes were seldom statistically significant.

_Atherosclerotic index_. Elastin tended to decrease with an increase in the severity of atherosclerosis. These negative correlations were most marked in the aortas and coronary arteries of White males and were here statistically significant (Table I).

_Other constituents_. In the aortas and coronary arteries negative correlations were observed between elastin and calcium. In White males these correlations were significant. In the cerebral arteries the correlations were positive. Similar correlations were present between elastin and total lipids.

_Vascular beds_. The concentration of elastin in the aortas was about double that in the coronary and cerebral arteries (Table I).

_Race_. With few exceptions significant racial differences were not obtained.

_Hexosamine_. Age. In the aortas and coronary arteries of White males hexosamine concentrations showed significant decreases with increasing age. In the corresponding vessels of Bantu hexosamine tended to increase with age. Hexosamine tended to increase with increasing age in the cerebral arteries of both racial groups (Table I).

_Atherosclerotic index_. In the aortas and coronary arteries of White males hexosamine concentrations tended to decrease with increasing severity of atherosclerosis, but tended to increase in Bantu. Hexosamine increased with increasing severity of atherosclerosis in the cerebral arteries of both racial groups (Table I).

_Other constituents_. In general hexosamine decreased in the aortas and coronary arteries of both races as calcium increased. In White subjects a similar decrease was noticed with an increase in the total lipids (Table I). In the majority of cases hexosamine, collagen and elastin showed mostly positive correlations in the aortas and coronary arteries of White males. In the cerebral arteries of both races hexosamine, calcium and total lipids were positively correlated.

_Vascular beds_. Aortas contained the highest mean hexosamine concentrations, followed by the coronary and cerebral arteries in that order (Table I).

_Race_. In certain age groups significant differences existed between the mean hexosamine concentrations of aortas and coronary arteries of Whites and those of Bantu. Significant race-related differences were not obtained in the cerebral arteries.

**DISCUSSION**

With increasing age there were distinct differences in the severity of atherosclerosis and the chemical composition of aortas and coronary arteries of both races as well as differences between Whites and Bantu. Racial differences were seldom demonstrated in the cerebral arteries. It was found in a previous study that gross atherosclerosis, as assessed by means of the atherosclerotic index,
showed only slight differences in the 2 races and sexes until about the third decade. Thereafter gross atherosclerosis increased more rapidly in the aortas and coronary arteries of White males.

Calcium increased in all 3 vessels of both races with increasing age, but as in the case of the atherosclerotic index, obvious racial differences were found in the aortas and coronary arteries, especially in the older decades. Anderson et al. have reported similar observations for the aorta. Neither the degree of the atherosclerosis nor the calcium concentration of the cerebral arteries differed to any extent in the 2 races. Since severe atherosclerosis was seldom seen in the aortas and coronaries of Bantu subjects or in the cerebral arteries of either race, the distribution of atherosclerosis would appear to be the general factor responsible for the pattern of distribution of calcium.

The calcium intake of Bantu is much lower than that of Whites. It may seem reasonable to regard low calcium intake and low serum-calcium concentration as factors contributing to the low calcium concentration found in aortas and in coronaries from older Bantu subjects. However, the dystrophic calcification occurring in human arteries is supposedly independent of serum-calcium level andWalker et al. could not demonstrate calcium deficiency in Bantu. The slightly lower serum-calcium level in Bantu is probably only a reflection of the lower serum-albumin level in this race. Hyperproteinemia with a normal parathyroid function is accompanied by low total serum calcium, but a normal concentration of diffusible calcium. Since it is presumably this ionic calcium that is the active fraction in calcification, a low total serum calcium associated with a normal ionic calcium concentration would not satisfactorily explain the lower vascular calcium in the Bantu.

It furthermore seems unlikely that a low calcium intake can be the explanation of the relatively low calcium levels found in the aortas and coronary arteries of Bantu in view of the fact that the cerebral arteries of White subjects also showed a relatively low calcium concentration, although the White subjects had a high calcium intake. The calcium levels in the different arteries of Whites therefore varied despite the fact that they were exposed to the same arterial blood.

Calcification of atheromatous lesions is currently regarded as a secondary process similar to calcification occurring in other tissues after necrosis. However, other local factors in the wall of the artery may also be important, since not all of the fibrous and necrotic plaques become calcified. Rosenheim and Robinson found that aortas from aged subjects calcified more easily in vitro than those from young subjects and that the uptake of calcium appeared to be higher at some sites than at others, although there was no mechanical stress or injury.

Blood-vessels naturally increase in size and weight with increasing age, and this increase is in part due to the formation of new connective tissue. Faber et al. and Noble et al. reported an increase in the collagen concentration of aortas with increasing age, while Anderson et al. noticed a decrease in the collagen concentration of aortas both with an increase in the severity of atherosclerosis and with an increase in age. In general our data support the observations of Anderson et al. The constantly low collagen levels in aortas of rural Bantu reported by these workers were not demonstrated in the present study. On the contrary, Bantu subjects tended to have higher collagen concentrations than White subjects. In the coronary arteries this difference was statistically significant. The higher collagen concentration in Bantu vessels was probably the result of the sligher degree of atherosclerosis present in Bantu subjects.

Elastin concentrations showed wide individual variations in the present study. Similar observations were reported by Pepler, who found that aortas showing no macroscopic changes differed considerably when examined microscopically. Data so far reported on the chemical concentration of elastin in blood-vessels and the changes that occur with increasing age and increasing severity of atherosclerosis are conflicting. Anderson et al. reported a decrease of the mean elastin concentration of aortas of White subjects with increasing age. This change was not apparent in Bantu. In the present study the elastin concentration decreased in the vessels of both Whites and non-Whites. The absence of marked racial differences in the 3 types of vessels is surprising in view of the fact that the White subjects showed more severe atherosclerosis, and some destruction of elastic tissues is said to accompany the atherosclerotic process. On the other hand, the results are in conformity with the observation of Pepler, who described degenerative changes of equal severity in the aortas of Bantu and White subjects.

The hexosamine concentration of a blood-vessel represents the combined hexosamine-containing material of the vessel, including acid mucopolysaccharides, mucoids and glycoproteins. Changes in the hexosamine concentration of blood-vessels, therefore, give no information about the individual components. Like collagen and elastin, the hexosamine content of the blood-vessels varied widely within each age group. This finding is in keeping with the fact that the patients died from a wide range of diseases.

In the present study the hexosamine levels of the aortas and coronary arteries of White subjects decreased with advancing age. Anderson et al. and Kirk et al. made similar observations. Buddecke reported an increase in the concentration of hexosamine with an increased severity of atherosclerosis, and similar results were reported for the aortas of Bantu by Anderson et al. In the present study an increase in hexosamine content with advancing atherosclerosis was found in the cerebral arteries of White subjects and in the aortas, coronary and cerebral arteries of Bantu subjects.

Buck demonstrated increased synthesis of sulphated polysaccharides in portions of rabbit blood-vessels showing fibroblastic activity, while no synthesis could be demonstrated in the devitalized necrotic areas. The low concentration of hexosamine observed in the aortas and coronary arteries of White subjects in the present study might therefore be a reflection of the relatively large areas of devitalized tissue present in the vessels of this group. On the same basis, the increase in hexosamine content of all
Bantu arteries and of the cerebral arteries of White subjects with advancing age would indicate the maintenance of fibroblastic activity with less severe degrees of atherosclerosis. The atherosclerotic indices and calcium levels of the various arteries in the two races support this view.

SUMMARY

The aortas, coronary arteries and cerebral arteries of 74 White subjects and 110 Bantu subjects between the ages of 10 and 69 years were analysed for their calcium, collagen, elastin and hexosamine contents.

The calcium concentration tended to increase with advancing age and increasing severity of atherosclerosis in both races. Beyond the age of 40 years this increase was considerably greater in the aortas and coronary arteries of White subjects than in those of Bantu subjects. The aortas and coronary arteries of White subjects had a higher calcium concentration than the cerebral arteries. Below the age of 40 years the calcium content was higher in the cerebral than in the coronary arteries of Bantu. Beyond the age of 40 years the mean calcium concentration of the aortas and coronary arteries of White males were significantly higher than those of Bantu.

The calcium concentrations of the cerebral arteries showed no statistically significant racial difference.

In general the collagen and elastin concentrations tended to decrease with increasing age, increasing severity of atherosclerosis and increase in calcium concentration. This negative correlation was especially noticeable in the aortas and coronary arteries of White subjects.

In White males the hexosamine concentration decreased in the aortas and coronary arteries with increasing age and with increasing severity of atherosclerosis. In the aortas and coronary arteries of Bantu and in the cerebral arteries of both races this trend was reversed.

Macroscopically, the aortas and coronary arteries of the 2 races showed significant differences in the degree of atherosclerosis. In general, the present chemical studies confirmed these racial differences. Racial differences were rarely found in the cerebral arteries.

REFERENCES