ON THE EFFECT OF ATHEROSCLEROSIS AND GOITRE ON AORTIC HISTOPATHOLOGY AND MUCOPOLYSACCHARIDE CONTENT

By

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ABSTRACT

The effect of goitre on aortic mucopolysaccharides in sclerotic and non-sclerotic subjects was studied by histological and biochemical methods. Atherosclerosis alone lowers the quantity of aortic acid mucopolysaccharides. Goitre alone shows a tendency to increase the mucopolysaccharide content of the aorta. Goitre accelerates the effect of atherosclerosis in decreasing the aortic mucopolysaccharides, i.e. fibrosing and sclerosing effect. Histological observations reveal that atherosclerosis is characterized, in all groups, by fatty degeneration of intima and inner elastic membrane and destruction of the elastic connective tissue, and in the process of repair the mucopolysaccharides increase. In connection with the ensuing collagenization, the mucopolysaccharides decrease. It is suggested that goitre by hypo- or hyperfunction causes metabolic disturbances in the aortic wall and thus promotes atherosclerosis.

Previously, it has been shown (Uotila et al. 1958) that goitre is much more common in subjects who have died from coronary sclerosis than in those who have died from other causes. From this, and from other circumstances, we arrived at the hypothesis that goitre may be aetiologically associated with atherosclerosis. The hypothesis appeared also plausible for the reason that the thyroid gland plays a part in controlling the mucopolysaccharide content and lipid metabolism of blood and tissues. With a view to further elucidation of the aetiological connections between goitre and atherosclerosis, we considered it appropriate to study, in human autopsy material, what effect atherosclerosis and goitre have on the mucopolysaccharide content of the aorta. The biochemical investigation was completed by histochemical investigations of the aorta.

The data bearing on the aortic content of mucopolysaccharides in athero-
sclerosis are controversial. *Dyrrbye & Kirk* (1957) report that there was a distinct decrease in the age group of 60–70 years as compared with younger groups. On the other hand *Kirk & Dyrrbye* (1957) state that no certain variation with age was observed in the total hexosamine, uronic acid, sulphate and protein contents of the isolated samples. Determinations of the amino sugar composition of the mucopolysaccharide material showed higher galactosamine and lower glucosamine content in samples derived from subjects of 60–70 years’ age than in samples from children and younger adults.

According to *Noble et al.* (1957), the connective tissue of atheromatous human intima shows an increase in collagen content and an increase in the binding of hexosamine with scleroprotein, i.e. collagen or elastin. However, the total hexosamine remains unchanged during the development of atheromatosis. *Buddecke* (1958) reports that atherosclerotic processes increase the ground substances as calculated from the total amino sugars. His separate analysis for glucosamine and galactosamine indicates that there is a shift of the glucosamine/galactosamine ratio within the general increase of hexosamines, glucosamine being preferentially increased. The elastin content decreases in parallel with the increasing severity of atherosclerosis but the changes in collagen content are not conclusive. Furthermore, formation of new connective tissue in cases of severe atherosclerosis may amount to 100 % of the normal quantity, whereas the lipid content only increases by 20–30 % of the aortic dry weight. According to *Moon* (1959), the degeneration of the inner elastic membrane accompanied by an accumulation of mucopolysaccharides and by fibroblastic proliferation represents the early, non-lipid phase of atherosclerosis in infants and adults. This is later followed by fibrous scar and degenerative lipid infiltration and calcification.

*Rinehart & Greenberg* (1951) by means of pyrodoxine deficiency, induced lesions resembling human atherosclerosis in monkeys. Metachromatically staining mucoid material accumulated in the intimal ground substance. *Mc Milllan & Weigensberg* (1957) were unable to detect, morphologically or chemically, any changes in acid mucopolysaccharide content in the aortas of rabbits with cholesterol-induced atherosclerosis. In similar experiments, *Gore & Barr* (1959) found histochemically that the earliest intimal lesions were characterized by lipids only, while mucopolysaccharides were not detected except in older lesions, mainly in the media. These findings were confirmed by means of chemical analysis (carbazol and orcinol methods) by *Bollet et al.* (1958).

**MATERIALS AND METHODS**

64 fresh aortic samples were obtained at autopsy. The subjects were all males, their age ranging between 40 and 60 years. The material was divided into four groups of 16 individuals each according to the size of the thyroid gland and the condition of the aortic wall. The first group (A) included cases with enlarged thyroid gland
(weighing over 40 g) and visible sclerosis of the aorta, the second (B) non-goitrous subjects with sclerosis, the third (C) goitrous, non-sclerotic cases, while the fourth group (D) consisted of accidental dead, non-goitrous and non-sclerotic individuals. Furthermore, some aortic samples from young subjects (16–20 years old), subjected to histological study, served as control series.

Biochemical determination of mucopolysaccharides

Only the ascending portions of the aorta were used. After removal of the adventitia, the intima-media parts were stored at —20°C in air-tight jars until their extraction. A weighed quantity from each aorta was extracted in the Bühler homogenizer, three times with acetone and once with ether. After filtering by suction, the aortic mass was dried in a vacuum desiccator over phosphorous pentoxide and thin slices of paraffine wax. The average dry substance quantity of fat-free tissue was 22.4 %; there was no difference between the four groups.

Isolation of the mucopolysaccharides was performed according to DyrbYe & Kirk (1957). Four samples, one from each group, were processed simultaneously so that errors due to unavoidable deviations in time schedule might be excluded. It was found that the precipitation of mucopolysaccharides in the different phases of the procedure was not complete after 12 hours but continued during several days.

The mucopolysaccharide content of the final solution was determined by the turbidimetric method of di Ferrante (1956), using commercial chondroitin sulphate from bovine nasal septa (Sigma Chemical Co.) as a reference standard.

Histological and histochemical investigation

The fresh sections were fixed for 24 hours in Lillie's buffered 10 % neutral formalin solution. The fixed tissue was dehydrated, cleared and embedded in paraffin. Serial cuts were made at about 10 µ and stained by the following methods:
1. Haematoxylin and van Gieson's stain,
2. Weigert's stain for elastic tissue,
3. Toluidine blue for metachromasia (Pearse 1960),
4. Lison's Alcian blue for mucopolysaccharides (Lison 1954),
5. The original periodic acid-Schiff technique by McManus,
6. Hyaluronidase extractions (Pearse 1960). As a testicular hyaluronidase, the preparation »Hyalase®« (Benger) was used. This purified testicular extract is available in ampoules each containing 1500 IU. Prior to use, a solution was prepared by dissolving the contents of each ampoule in approximately 3 ml of 0.85 % saline. As a bacterial hyaluronidase, »Hyason«® (Organon) was used. The contents of each ampoule of this product (150 T. R. U.) we dissolved in 1.5 ml of 0.85 % sodium chloride solution. After three hours' incubation at 37°C in a solution of one of the above-mentioned preparations, the washed sections were stained for 20 minutes in 0.5 % aqueous toluidine blue and compared with identically stained control sections which had been incubated for three hours at 37°C in saline only,
7. Formol-fixed frozen sections were stained for fat by the Sudan method,
8. The sections were furthermore studied for the distribution of minerals by micro-incineration. The technique has been described by Hintzsche (1956). Paraffin sections were cut at 5 µ, placed on slides with the aid of liquid paraffin, and incinerated at 520°C for one hour, after the heat had been brought up gradually during three hours. The incinerated preparations were examined by darkfield microscopy, when white granules represent the minerals.
RESULTS

a) Histopathology

The results of the histological investigation are presented in the following, grouped on the basis of the development occurring with increased age.

As a kind of normal or control state one may consider the following condition encountered in subjects under 20 years. The aorta is covered by an endothelium with a very thin underlying intima. There may be some few sudanophilic grains or drops in its fibrocytes. The intima contains acid mucopolysaccharides in some quantity and its ash contents on microincineration is scanty. On elastin staining, the inner elastic membrane is clearly delineated; no fat is found in it, but there are mucopolysaccharides in some quantity; its ash content is moderate. In the media, the elastic fibres together with the muscle cells form a network showing mucopolysaccharides in some quantity in its interstices and scanty amounts of fat in the fibrocytes. The media is fairly rich in ash. In the adventitia, elastic and, particularly, collagenous connective tissue and some mucopolysaccharides are encountered. The ash content is less than in the media.

In the first, or degenerative phase of atherosclerosis, the intima is thickened; its fibrocytes or fibroblasts contain sudanophilic substance in greater quantity and the quantity of mucopolysaccharides in the intima is increased. The inner elastic membrane may be in good condition but it is usually frayed, fragmentary and indistinct in structure, and frequently markedly sudanophilic. The quantity of mucopolysaccharides is increased in the region of the membrane and around it, in the region of intima and media. The ash content is usually lowered in the area of elastic destruction. In the region of the inner layer of the media the changes are akin to those in the region of the inner elastic membrane.

In the second, or regenerative phase of atherosclerosis, the degenerative processes described in above continue but at the same time the regenerative processes, which have already partially begun in the preceding stage, gain in emphasis. Delicate collagen fibres grow in the region of the intima, and such fibres, in greater amount, are seen in the area of elastic membrane and the inner third of the media. The matter is thus one of scarry collagenization, which was already preceeded by the increase in mucopolysaccharides becoming less with increasing collagenization or sclerosis.

The third phase of atherosclerosis, again, is mainly degenerative although some regeneration also occurs. The foci rich in fat or collagen in the intima show a tendency towards necrobiosis and necrosis, giving rise to atheromatous plaques. In their vicinity, the degenerative changes of the inner elastic membrane and of the inner third of the media are also distinctly observable. The atheroma is usually markedly sudanophilic and its surroundings are rich in mucopolysaccharides and later become collagenized. The ash content of the atheroma is comparatively high as a rule due to calcium.
It may be mentioned, moreover, that testis hyaluronidase digestion abolished almost all metachromasia of our aortic specimens. After bacterial hyaluronidase digestion, the metachromatic reaction was almost as marked as without it. Therefore it is evident that the aortic mucopolysaccharides are mostly chondroitin sulphates. This is consistent with the results reported in Pearse's (1960) book.

b) Content of mucopolysaccharides

The quantities of mucopolysaccharides isolated from each specimen are shown in the accompanying table. (Table 1).

Statistical treatment by one-way variance analysis and criticism of the results with the aid of Tukey's test reveal that there are no statistically significant differences between the groups except that the difference between groups A and C is statistically almost significant ($P = 0.1$). This would seem to indicate that goitre in association with atherosclerosis lowers the quantity of aortic mucopolysaccharides, as compared with the aorta of goitrous subjects.

Table 1.
Aortic mucopolysaccharides (mg/g fresh tissue).

<table>
<thead>
<tr>
<th>Specimen No.</th>
<th>Goitre + Sclerosis + group A</th>
<th>Goitre — Sclerosis + group B</th>
<th>Goitre + Sclerosis — group C</th>
<th>Goitre — Sclerosis — group D</th>
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<tbody>
<tr>
<td>1</td>
<td>2.19</td>
<td>1.70</td>
<td>1.45</td>
<td>1.68</td>
</tr>
<tr>
<td>2</td>
<td>1.98</td>
<td>2.06</td>
<td>3.95</td>
<td>1.55</td>
</tr>
<tr>
<td>3</td>
<td>1.86</td>
<td>2.00</td>
<td>3.06</td>
<td>3.02</td>
</tr>
<tr>
<td>4</td>
<td>1.57</td>
<td>2.21</td>
<td>2.23</td>
<td>1.87</td>
</tr>
<tr>
<td>5</td>
<td>3.39</td>
<td>1.78</td>
<td>2.54</td>
<td>1.64</td>
</tr>
<tr>
<td>6</td>
<td>2.12</td>
<td>2.66</td>
<td>1.56</td>
<td>2.45</td>
</tr>
<tr>
<td>7</td>
<td>1.86</td>
<td>1.95</td>
<td>2.00</td>
<td>2.89</td>
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<tr>
<td>8</td>
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<td>2.40</td>
<td>2.12</td>
<td>3.30</td>
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<tr>
<td>9</td>
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<td>5.05</td>
<td>3.93</td>
<td>4.01</td>
</tr>
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<td>3.25</td>
<td>1.98</td>
<td>4.00</td>
<td>3.75</td>
</tr>
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<td>2.10</td>
<td>1.94</td>
<td>3.23</td>
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<td>12</td>
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<td>2.52</td>
<td>2.57</td>
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<tr>
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<td>2.18</td>
<td>2.62</td>
<td>1.85</td>
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<tr>
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<td>7.94</td>
<td>7.07</td>
<td>9.85</td>
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<td>16</td>
<td>4.98</td>
<td>5.90</td>
<td>6.68</td>
<td>5.30</td>
</tr>
</tbody>
</table>

Mean 2.92 3.32 3.78 3.38
If the groups A and B, including all atherosclerotic cases, are combined (group A1), the average mucopolysaccharide quantity will be 3.15 mg per g. Combination of the non-sclerotic cases to form one single group (group A2 = groups C plus D) gives an average mucopolysaccharide content of 3.58 mg per g for this group. There is statistically almost significant difference ($P = 0.05$) between these values, which indicates that atherosclerosis reduces the amount of aortic mucopolysaccharides.

Combination of the goitrous cases (groups A plus C = group B1) gives an average mucopolysaccharide content of 3.34 mg per g, similar combination of the non-goitrous cases (groups B plus D = groups B2) resulting in the figure of 3.36 mg per g for this content. The two values are identical.

Furthermore, on separation by means of two-day variance analysis of the effects exerted upon the mucopolysaccharides by sclerosis and goitre, interaction between the two was established with 90 per cent confidence ($P = 0.1$). This indicates that the combination of atherosclerosis and goitre lowers the quantity of mucopolysaccharides to a lower level than is consistent with the average individual effects of goitre and atherosclerosis. Moreover, goitre in the absence of sclerosis may produce values higher than those to be expected on the average. On judging these results, one should take into account the comparatively wide dispersion in age of our cases (40–60 years) and the fact that despite the macroscopic homogeneity of the groups, the microscopic findings were fairly variable within each group. We think therefore that the significance of our results is higher than is indicated by pure statistical treatment.

On the basis of the above, we consider the following conclusions to be justified: Atherosclerosis alone reduces the quantity of acid mucopolysaccharides in the aorta. Goitre alone would seem to increase the quantity of mucopolysaccharides in the aorta. In spite of this, goitre potentiates the lowering effect of atherosclerosis upon the aortic mucopolysaccharides.

**COMMENTS**

Above all, histopathological and histochemical observations indicate that atherosclerosis is a degenerative phenomenon, to which the wall of the blood reacts by reparative changes. It does not seem possible to explain the fatty degenerations and destruction of the elastic tissue of the aorta in any other way. Already under normal conditions the fibrocytes of the intima appear to contain fat droplets in a scanty amount similar to any other cells of the organism. However, it is thought that their occurrence in greater than normal abundance has to be considered a degenerative phenomenon, all the more so as it seems to be temporally and locally associated with the elastic destruction of the inner elastic membrane and the inner layers of the media.

The acid mucopolysaccharides of the aorta form one of its normal con-
stituents as a result of the metabolism of the fibrocytes. Their occurrence in excess of normal is obviously a reactive change, which may be degenerative, as it is probably in idiopathic medionecrosis of the aorta (Raekallio 1958), or regenerative as in the healing of connective tissue in general. We have no certain knowledge as to which applies in atherosclerosis, but in part at least the change must be regenerative. This is already indicated by the fact that the acid mucopolysaccharides usually increase in the region surrounding degenerative elastica destructions and atheromata.

From the histopathological point of view, degeneration and increase in mucopolysaccharide content are followed by scarry collagenization, and the quantity of the mucopolysaccharides decreases. The findings are supported by the fact that we have also established a decrease of the mucopolysaccharides in connection with atherosclerosis by biochemical means. This finding would also seem consistent with the well-known fact that the mucopolysaccharides frequently increase in the connective tissue in the process of new formation but decrease on fibrosis. The explanation for the results of some investigators, which disagree with the above, may perhaps be sought in methodical differences and possibly also in differences attributable to the material. As regards the elevated mucopolysaccharide values found by Buddecke (1958) in sclerosis, they are based on determinations of the total hexosamine and are misleading in that only part of the hexosamines of the connective tissue occur in mucopolysaccharides (Dyrbye & Kirk 1957; Boas 1955), while the rest are incorporated in uronic acid-free mucopolysaccharides. The forcible extraction procedure employed by Buddecke will obviously also set free from the tissue the hexosamine which is bound to proteins.

One of our observations possesses fundamental significance, namely, that goitre increases the mucopolysaccharide-lowering effect of atherosclerosis. This can probably also be stated by saying that goitre promotes the fibrosing effect of atherosclerosis. This result, apparently, provides confirmation of our previous observation concerning the highly significant coincidence of goitre and coronary mortality and at the same time offers an idea of the causes responsible for this coincidence.

The question of the character of the promoting effect exerted upon atherosclerosis by goitre is rather more complex. However, from numerous clinical and experimental studies it is known that hypothyreosis promotes atherosclerosis. It is also known that thyrotropin increases the hyaluronic acid content of the connective tissue (Asboe-Hansen 1950). In our Institute, Perttala (1960) has established that the feeding of cholesterol to fledgling roosters increases the $^{35}$S uptake of the aorta, as does prolonged feeding of thiouracil. Thyroidin alone does not promote the $^{35}$S uptake to any appreciable extent but both thiouracil and thyroidin potentiate the $^{35}$S uptake-increasing effect of cholesterol. Small doses of thyroxin intensify cholesterol-induced atherosclerosis.
It is probable that both hypo- or hyperfunction of the thyroid cause metabolic disturbances of aortic or arterial wall which accelerate its »normal« degeneration. Since the goitre endemic in Finland has some hypothyroid features (Hortling & Hiisi-Brummer 1959) but also a definite tendency to toxicity (Wahlberg 1938) this might explain the atherosclerosis-promoting effect of goitre.

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