CONSIDERABLE emphasis has been placed upon the role of lipid abnormalities in the pathogenesis of atherosclerosis, but little attention has been paid to the possible significance of deranged tissue metabolism. In many species, including man, the earliest atheromatous lesions are characterized by the subintimal accumulation of a mucoid material. These mucopolysaccharide deposits were described by Taylor in 1953. Faber suggested that such mucoid substances were directly concerned with the deposition of cholesterol in tissues, including the aorta, and this view was later expanded by Altshuler and Angevine. The possible significance of altered mucopolysaccharide metabolism in the genesis of atherosclerosis was recently stressed by Wartman, while Schwartz et al. have demonstrated that similar histochemical abnormalities occur at the sites of aortic atheromatous lesions in the cholesterol-fed rabbit, together with an elevation of the serum hexosamine levels.

The ground substance of the arterial tree contains chondroitin-sulphuric acid compounds, but the presence of hyaluronate is in doubt. Depolymerization or hydrolysis of these materials leads to an elevation within the serum of their component amino sugars. This will occur in rabbits after a solitary injection of testicular hyaluronidase and after prolonged administration of the substance. A similar elevation in the serum hexosamine levels of rats was produced by Schwartz and Gilmore and in addition marked elevations of the serum mucoproteins were noted. These findings suggest that elevations of both the amino sugar and the serum mucoprotein fractions may be interpreted as reflecting a breakdown of mesodermal ground-substance mucopolysaccharides.

One might expect that, as the histochemical abnormalities of human atherosclerosis probably reflect changes in both the quantity and state of polymerization of arterial mucopolysaccharides, these alterations might be reflected by changes in the serum amino sugar and mucoprotein levels.

This paper describes the results of an investigation of the effects of age and atherosclerosis on the serum hexosamine and serum mucoprotein levels in man.

MATERIALS AND METHODS

Three groups of subjects were investigated. The first group consisted of 24 normal male medical students with ages varying from 19 to 35 years (average 25 years). The second group consisted of 23 atherosclerotic male patients with an age range of 40 to 72 years (average 56 years). Most of these patients were suffering from angina of effort; 3 had myocardial infarction 6 months or more prior to investigation from which they had clinically recovered. The third group of 16 were clinically nonatherosclerotic men with an age range of 37 to 71 years (average 52 years). The ages of these latter individuals were comparable with those of the atherosclerotic group. These age controls were normotensive, without clinical or electrocardiographic evidence of myocardial ischemia, and judged to be free from atherosclerosis on clinical assessment. Single venous blood samples were collected from the median cubital veins.
TABLE 1.—Changes with Atherosclerosis and Age in the Serum Mucoprotein and Hexosamine Levels

<table>
<thead>
<tr>
<th>Atherosclerotic subjects</th>
<th>Age controls</th>
<th>Young controls</th>
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<tr>
<td>Serum mucoprotein (mg./100 ml.)</td>
<td>Serum hexosamine (mg./100 ml.)</td>
<td>Serum mucoprotein (mg./100 ml.)</td>
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<td>Age: (yrs.)</td>
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Means

56 119.0 142.5 52 97.1 95.6 25 92.2 86.6

S.E.M

±0.6 6.33 ±1.99 ±2.81 ±1.33 ±2.61

*p <0.001 <0.01 <0.05 <0.02 — —

* Probability was assessed by the student’s t test, except in the case of the mucoprotein levels in atherosclerosis where a Fisher-Behrens test was applied, because of a difference in the standard errors of the 2 groups.

SCHWARTZ, GILMORE

and the sera were obtained after centrifugation at 3,000 r.p.m. for 10 minutes. The biochemical determinations were performed without knowledge of the source of the samples.

Serum hexosamine levels were determined by the method of West and Clarke on 0.2 ml. of serum. The pink color developed was read in a Unicam S.P. 600 spectrophotometer at 540 mμ. Serum hexosamine was expressed as mg. per 100 ml. of glucosamine with a standard of glucosamine hydrochloride.

Serum mucoprotein was determined according to the method of Winzler et al. with certain modifications. Only 0.2 ml. of serum was used and the mucoprotein was determined as protein by the sensitive method of Lowry et al. with the Folin-Ciocalteau reagent. Mucoprotein was then expressed as mg. per 100 ml. of serum in terms of its protein content using human albumin standards. The blue color developed was again read in a Unicam S.P. 600 spectrophotometer at 760 mμ.

RESULTS

An elevation of the serum mucoprotein with age was found, the mean level in the student group being 86.6, while the mean of the non-atherosclerotic age controls is higher (95.6) (p < 0.02). A greater difference between the atherosclerotic and nonatherosclerotic groups can be readily seen from table 1, where the mean level for the former is 142.5, and for the latter 95.6. This elevation of the serum mucoprotein level in atherosclerosis above the level in a nonatherosclerotic group of comparable age is highly significant (p < 0.001). Similar results were obtained with respect to serum hexosamine differences. The increase in the mean level with age from 92.2 to 97.1 in the nonatherosclerotic age controls is significant (p < 0.05). In the atherosclerotic group the mean level (119.0) is significantly higher than the mean level of the age control group (p < 0.001). From a comparison of the mucoprotein and hexosamine values it can be seen that the degree of change is greater with the former than the latter.

DISCUSSION

The findings of an increase in metachromatic staining material in the lesions of human atheroma by Taylor suggested to the authors that mesodermal ground-substance changes may be involved in the pathogenesis of atherosclerosis. It has further been demonstrated that similar histochemical changes occur in cholesterol-fed rabbits, together with a concomitant elevation of the serum amino sugar.

*Glucosamine hydrochloride, kindly supplied by Nutritional Biochemical Corporation, Cleveland, Ohio.
EFFECT OF ATHEROSCLEROSIS AND AGE ON SERUM LEVELS

levels. These findings, histochemical and biochemical, both suggest that ground-substance mucopolysaccharide depolymerization occurs in the pathogenesis of atheroma in cholesterol-fed rabbits. A similar elevation in the serum amino sugar levels in cholesterol-fed rabbits has been reported.12

Accentuation of the depolymerization by testicular hyaluronidase has been shown to increase atherogenesis in hypercholesterolemic rabbits.5, 13, 14 Contrasted with this finding is the inhibition of atherogenesis in cholesterol-fed rabbits with cortisone.15-17

In this paper, biochemical evidence of an increased breakdown of ground-substance mucopolysaccharides in male patients with clinical evidence of atherosclerosis is provided by the finding that both the serum mucoprotein and hexosamine levels in the atherosclerotic group are significantly elevated above those of the nonatherosclerotic age controls (table 1) (p < 0.001).

In order to determine more exactly the significance of serum mucoprotein elevations, Schwartz and Gilmore8 produced an in vivo enzymatic depolymerization of ground-substance mucoids in rats with testicular hyaluronidase. Marked elevations in both the mucoprotein and hexosamine fractions were recorded. Similar elevations in the serum hexosamine levels of rabbits have been reported by this specific enzymatic depolymerization of ground substance.5, 7 One may therefore interpret some elevations of both these fractions as reflecting a breakdown of connective tissue mucopolysaccharides. Further support for this interpretation is found in the elevations of the serum glycoproteins that have been reported in the so-called collagen diseases, rheumatic fever,18 and in the rheumatic group of disorders.19 The similarity between the biochemical findings in atherosclerosis as reported in this study and those found in the collagen diseases is striking, though the changes in atherosclerosis are of lesser degree. Similar observations on the glycoprotein levels in atherosclerosis have been recently reported by Antonini and Salvini20 while this work was in progress.

Metachromatically staining intercellular substance is probably involved as a precursor in the formation of reticulum and collagen fibrils in granulation tissue.21-24 It is possible therefore that the extensive intimal and subintimal fibrosis that characterizes established atheromatous lesions in both the experimental animal and in human subjects is derived from the metachromatically staining mucopolysaccharides seen in similar sites at an earlier stage in these lesions. Of related interest is the observation of Watson and Pearce25 that in pretibial myxedema progressive fibrosis is associated with diminution of tissue mucopolysaccharide concentration.

If such a process of connective tissue ground-substance depolymerization is of significance in atherogenesis, it is of interest to note the low mucoprotein levels in an ethnic group, the nomadic Australian aborigine, which has a low incidence of atheroma. The low serum glycoprotein level of this group is contrasted with the higher level that occurs in a group of urban aborigines, whose levels do not differ significantly from those of normal white Australians.26

A trend toward an increase with aging in both the glycoprotein fractions is not surprising. Atherosclerosis has a greater incidence in the older age groups, and an increased ground-substance catabolism due to age would possibly help account for this. The possibility of some occult atherosclerosis in the control group cannot be excluded in a community such as this, in which atherosclerosis is becoming a common disorder.

The present findings suggest that depolymerization of the mesodermal ground substance plays a role in the pathogenesis of human atherosclerosis. However, whether it is basic to the pathology of atherosclerosis or merely occurs secondary to some other process, such as hypercholesterolemia, still remains to be determined.

Summary

Highly significant elevations of both the serum mucoprotein and hexosamine levels in atherosclerosis are reported. There is also a
trend for an increase in both these fractions as the result of aging.

These findings are interpreted as reflecting an increased depolymerization of connective tissue ground-substance mucopolysaccharides. It is suggested that connective tissue ground-substance breakdown plays a role in the pathogenesis of atherosclerosis, possibly as a precursor to fibrogenesis.

ACKNOWLEDGMENT

We are indebted to Professor Sir C. Stanton Hicks for invaluable advice and criticism; and to Professor J. S. Robertson. Also to Mr. Graham Constantine for advice and Mr. J. A. Peters, D.M.T., for technical assistance; and to all the patients and students for their willing cooperation in this investigation.

SUMMARIO IN INTERLINGUA

Es reportate significativissime elevationes del nivellos seral de mucoproteina e de hexosamina in patientes con atherosclerosis. Il existe etiam un tendienta accrescitori in ambe iste fractiones como effecto de avantiamento del etate.

Iste constatationes es interpretate como reflexion de un augmentate dispolymerisation del mucopolysaccharidos in le substantia basal del histos conjunctive.

Es suggerite que discomposition del substantia basal in histos conjunctive ha forsas un rolo in le pathogenese de atherosclerosis e es possibilemente un precursor de fibrogenese.

REFERENCES

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A technic is described for the use of intraocular arterial homotransplantation for the study of spontaneous or experimentally induced atherosclerosis in animals. A portion of an artery is excised and a small piece of normal or atherosclerotic vessel is implanted through an incision into the anterior eye chamber. The transplant is moved to the opposite side across the chamber and wedged between the cornea and iris, with the intimal surface facing outward. At a selected time, a host animal may be sacrificed and histologic section may be studied to determine the nature and degree of both structural and biochemical changes. The adventitia of the transplants becomes attached to the anterior surface of the host's iris by fibroconnective tissue in less than 8 days. In addition, the thickened intima of atherosclerotic transplants becomes extensively vascularized, but that of the normal transplants does not. Thus far, transplants have been in place for 6 months, and this would seem to permit the use of these animals in relatively long-term nutritional and pharmacologic experiments.
Effect of Atherosclerosis and Age upon the Serum Mucoprotein and Hexosamine Levels in Man
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