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Mucopolysaccarides, «Clearing Factor», Age, and Arteriosclerosis

By **Francesco Mario Antonini** and **Lino Salvini**

If we analyze the plasma of atherosclerotic individuals, either chemically by determination of the cholesterolemia, the blood concentration of total lipids, phospholipids and C/P ratio, or by chemicophysical technique (ultracentrifugation, electrophoresis, precipitation by Cohn's method), important variations are frequently observed as much in the chemical as in the chemicophysical composition of plasmatic lipids. We shall not dwell upon the importance of determining cholesterol, total lipids, phospholipids and C/P ratio. This has already been discussed at length, and has for some time been introduced into medical practice. We shall merely record that, although these are indications of some worth for the bigger alterations in lipid metabolism, they are only clearly demonstrative in a small number of cases. But too many atherosclerotic subjects escape diagnosis with these chemical determinations, and it is undoubtedly for this reason that the criticism of the medical practitioner in this regard is at least partly justified.

The demonstration that cholesterol and the greater part of lipids are not free as such in the plasma, but linked in lipoprotein complexes, and the development of suitable techniques for the study of lipoproteins, has enabled *Gofman* et al. (1, 2) to bring a notable contribution to the knowledge of the relations between lipids, understood as lipoproteins, and atherosclerosis.

On the whole, *Gofman* has demonstrated statistically that, while cholesterolemia may only be increased in a limited number of atherosclerotics, the lipoproteins studied by ultracentrifugation display considerable modifications in normal composition, even when the blood cholesterol is within normal levels.

If instead of studying only the alterations of certain fractions of the β group (with *Gofman* technique) we try to extend our study to all lipids, as can be achieved with electrophoresis on paper, it will be observed that

the alterations are not confined solely to the Sf 20–400 β lipoproteins, but extend to all the lipoprotein fractions, since, beside an accumulation of lipids in β lipoproteins, a relative and absolute reduction is found in the lipids contained in α globulins.

Although in the lipoprotein diagram it is possible to separate 5 or 6 fractions, in practice it is more convenient for reproduction of the results and better disclosure of the essential alterations of the lipoprotein derangement present in the atherosclerotic, to limit examination to the relation between α and β lipoprotein fractions. The β/α ratio is an immediately apparent test, directly indicating the state of dispersion of the lipids conveyed by the blood – a higher ratio corresponding to a prevalence of less dispersed lipids, and a lower ratio to an increase of the more dispersed lipids. Increase in the β/α ratio signifies a preponderance of lipoproteins with lesser electrophoretic mobility, lower protein content, lower density and greater molecular size, roughly corresponding to *Gofman's* high and very high Sf lipoproteins (Sf 10–400), in respect to lipoproteins with opposite characteristics (α).

We have for some time insisted upon the utility of this ratio (3–7), which in our ample case list has proved to be perfectly coherent with diagnostic requirements, and is now generally used by many researchers. Certainty as to the advantage of this determination has been given by the large number of definitely atherosclerotic cases collected by us in recent years (over 3000, comprising atherosclerotics and normal subjects of different social conditions and dietetic habits, divided into age and sex groups, in which, besides determination of cholesterol, total lipids, phospholipids and C/P, we regularly examined the serum proteins and lipoproteins during fasting). Instead of showing in detail the results which were later confirmed by authors in Italy and abroad, let us briefly sum up the main conclusions supported by solid statistics (5, 6).

While cholesterol, the phospholipids and C/P ratio have proved scarcely significant in distinguishing atherosclerotic from normal individuals of the same age and sex, the β/α ratio has been found to be very significantly increased in truly atherosclerotic subjects (infarct or peripheral circulatory lesions of certain atherosclerotic nature).

The statistical significance which we observed in our cases was far superior to that which *Gofman* was able to disclose by the more complex determination with ultracentrifugation of Sf 10–20. This is understandable when bearing in mind that with the electrophoretic picture, we control not only the alterations occurring in the β, but the equally important alterations observed within the sphere of the α lipoproteins. *Gofman's* metabolic blockage could also be interpreted as an incapacity of α

lipoprotein complex to form, which, owing to their chemicophysical characteristics, normally migrate with the velocity of α_1 proteins. If, in an ample number of cases and on a strictly statistical basis, we follow the variations in the β/α ratio at the different ages and in the two sexes, we shall see that while there are very low values at young ages both in men and women, during the thirties a clear increase in β/α takes place in men as compared with the values presented by women (Fig. 1). These variations tell us that men tend towards alteration earlier than women,

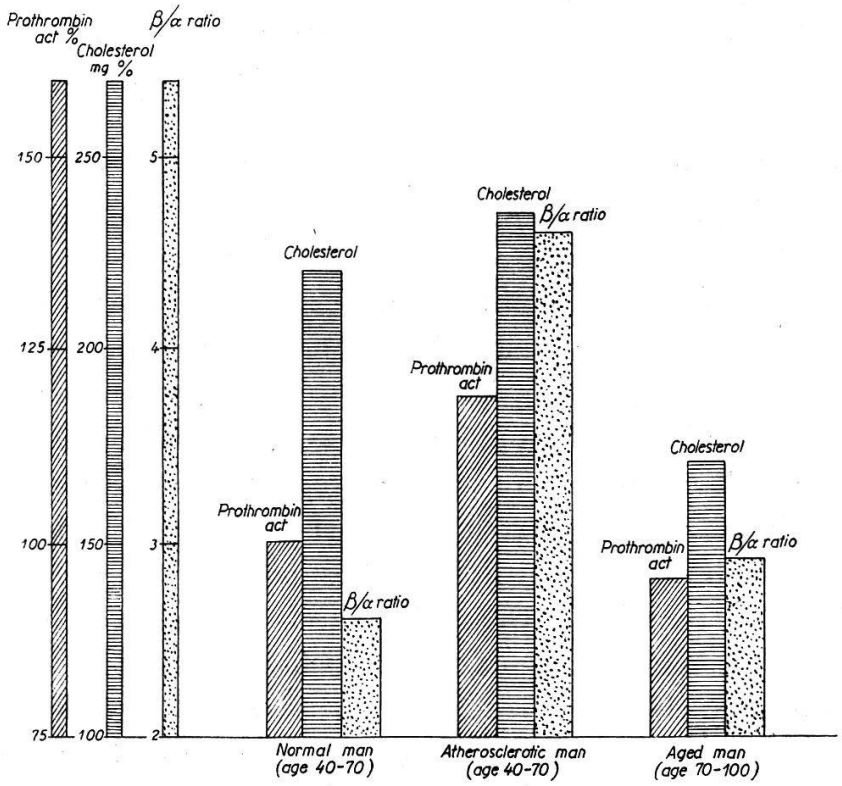

Fig. 1. The columns represent medium rates observed in normal, atherosclerotic subjects and in senility (123 subjects).

	Medium values	S.D.
Normals 40–70 years		
Prothrombin activity %	100	–
Cholesterol mg %	220	± 41.5
β/α lipoproteins	2.65	± 1.02
Atherosclerotics		
Prothrombin activity %	118	–
Cholesterol mg %	223	± 50.1
β/α lipoproteins	4.5	± 1.12
Aged 70–100 years		
Prothrombin activity %	95	–
Cholesterol mg %	174	± 35.3
β/α lipoproteins	2.7	± 1.04

Relation between normals and atherosclerotics 40–70 years
Cholesterol $t = 0.42$, $P > 0.05$
β/α lipoproteins $t = 3.54$, $P < 0.01$

and this corresponds to what is observed in clinical and anatomico-pathological practice, for the atherosclerotic lesions already appear in men at an early age, while these alterations are only exceptionally seen in women while the sexual life is flourishing, although they may be encountered in women who have been prematurely castrated.

Every time the humoral alterations clearly exceed the average levels established for individuals who are apparently healthy for their age and sex, we must suspect atherosclerotic lesions. We should however remember that, although relatively constant, the lipoprotein alterations sometimes present violent changes which are apparently not easy to explain. Clearly normal tracings may be found in definitely atherosclerotic subjects.

This contrast is frequently observed in elderly patients, even with severe atherosclerotic lesions.

It is not to be excluded that alteration in the lipoprotein situation, as also the setting in of the anatomical lesion, may be a discontinuous process in «thrusts», caused by enzymatic, hormonal mechanisms, of which the phenomenon of menopause may be an example. At any rate it is necessary to appraise the results of the lipidogram taking age and sex into account, and it is above all advisable to carry out repeated examinations in successive periods of time in correlation with other tests, which, even if less sensitive (cholesterol, total lipids, phospholipids), can complete the necessary humoral picture for the early diagnosis of atherosclerosis.

Among the mechanisms regulating the chemicophysical situation of lipids, we have principally to record heparin which, as we have seen by activating the clearing factor, tends to normalize any alterations in the distribution of lipoproteins, facilitating the conversion of the large β group lipoprotein molecules into the smaller one of the α group. But more than reorientation of the lipoprotein structures, we believe that a basic demonstration of the rôle played by heparin in the pathogenesis of atherosclerosis is provided by the assay of heparinoid substances (according to *Gibson* (8) in the blood of atherosclerotic subjects. If applied to an ample number of cases, this method provides statistical foundations. In the atherosclerotic, the heparinoid substances have proved to be clearly reduced—so significantly as to lead us to believe that this reduction may be intimately connected with the pathogenesis of atherosclerosis.

It will be seen from our statistics (Fig. 2) that, in the subjects examined by us, a low concentration of heparinoid substances in the plasma coincides with an increase in the β/α ratio, thus causing us to believe that a low quota of heparin is responsible for the lipoprotein alterations. Any doubt that the heparin in the blood might not be assayed by this method,

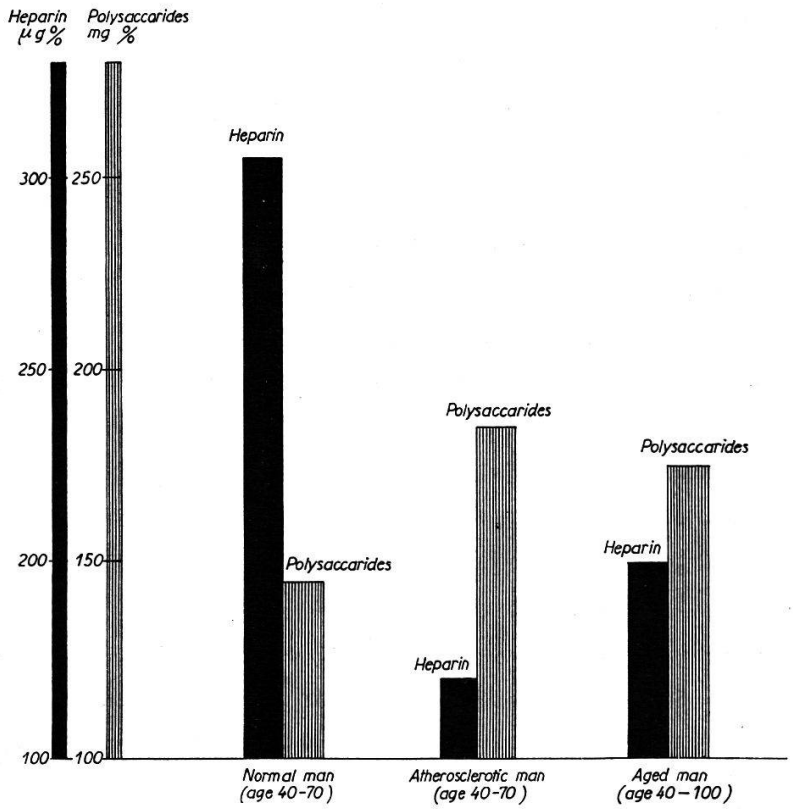

Fig. 2. The columns represent medium rates observed in normal, atherosclerotic subjects and in senility (123 subjects).

	Medium values	S. D.
Normal 40–70 years		
Heparinoid substances γ %	300	± 145
Protein polysaccarides mg %	145.6	± 20
Atherosclerotics		
Heparinoid substances γ %	136	± 72
Protein polysaccarides mg %	182	± 29
Aged 70–100 years		
Heparinoid substances γ %	200	± 120
Protein polysaccarides mg %	180	± 30
Relation between normal and atherosclerotic subjects		
Heparinoid substances t = 5.43, P<0.01		
Protein polysaccarides t = 5.40, P<0.01		

has been dissipated by the further experiments of *Engelberg* (10), who demonstrated chromatographically and biologically that the substance isolated by this method is at least partly heparin. *Engelberg* himself also observed that there is an inverse ratio between blood concentration of heparin and the lipoprotein situation which he examined by means of *Gofman's* ultracentrifuge. It should however be borne in mind that not only does heparin possess the chromotropic property of metachromasia, but there are numerous other substances of polysaccharide nature in the human organism which have the property of changing the basic stains of aniline, toluidine blue, to violet-red. These are chemical substances

originating mainly in the connective tissue. The decrease of the metachromatic substances of the blood might therefore be an indication of that yielding of the mesenchymas which many assert as being at the root of diseases of senility. In actual fact, determination of the number of the mast cells in the connective tissue of some organs in atherosclerotic individuals, demonstrates a considerable reduction in the number of these elements as compared to normal. Moreover, the animals in which atherosclerosis is most easily provoked are those less provided with mast cells, while the better provided animals are difficult to render atherosclerotic.

The conception that a deficit of heparin and "clearing factor" may be at the root of the humoral alterations present in atherosclerosis, is based on these established facts:

1. Clarification of the lipemic plasma induced by heparin (11).

2. Reorientation of the lipoprotein molecules, with transfer of lipids from the slower fractions to the quicker and more stable (12).

3. Diminution of the mast cells and of the heparinemia in atherosclerotic and arteriosclerotic subjects (13).

4. Protective effect of heparin upon atherosclerosis by cholesterol in the rabbit (14, 15).

5. Decrease of "clearing factor" in atherosclerotic, arteriosclerotic subjects and in old age (58).

We should however like to state that one may find a discordance in the relations between lipoproteins and heparinemia in the very elderly person, since often, against remarkably low levels of heparinoid substances, a high β/α ratio may not be found. Why, then, if heparinemia generally governs the onset of lipoprotein modifications, do we not find in the elderly hypo-heparinemic an increase in the pathological lipoproteins, but rather that the cholesterol and total lipids have diminished in parallel? This behaviour is probably connected with a profound reduction in endogenous synthetic processes, and contemporaneously with diminished intake of exogenous lipids, whether due to lesser introduction or lesser absorption. The reduction of heparin, which can have such a strong influence on the conveyance of lipids in an organism subjected to a diet particularly rich in fats, or in which the synthetic processes are considerably excited, may not be important when the lipids to be metabolized are considerably reduced.

Conversely, an over-production of heparin may explain the more balanced lipoprotein situation in the young woman compared with a man of the same age. Here the hormone influence must play a decisive rôle, since it seems from many researches that during the menstrual

cycle an increase in heparin takes place, mainly in the folliculinic period (7–10). At the same time it is known that the administration of estrogens can normalize hypercholesteremia and altered lipoprotein distribution; while androgens would aggravate the humoral picture of atherosclerosis (16, 17). Our recent observations enable us to state that adequate doses of estrogen are capable of considerably increasing the release of heparinoid substances from the tissues, simultaneously with increase in the blood concentrations of glucosamine and non-glucosaminic polysaccharides. The possibility that estrogens stimulate the formation of metachromatic substances may be linked with their more general influence in stimulating the mesenchyma. If the production of heparin is actually connected with the number and activity of the mast cells, any stimulus will be capable of influencing the production of this substance. Certain still unknown deficiences, lack of organizers, enzymatic and hormonal defects, prolonged toxic stimuli, and lastly a particular diathesis on a hereditary background, could explain the prematurely diminished activity of cells producing heparin, earlier in the atherosclerotic than in the normal individual. A diet rich in lipids could be one of the more important exogenous causes: we have found that a clear diminution is to be observed in the circulating heparinoid substances after a fatty meal. A prolonged over-fatty diet in predisposed individuals could in time lead to exhaustion of the cells forming heparin (19, 20).

Just as over-fatty diet has great importance in atherogenesis, so it has been ascertained that a diet deficient in calories and fats, especially animal, tends to normalize the lipoprotein alterations in the atherosclerotic.

Naturally, among the dietetic factors responsible for lipemia we must include all those substances capable of facilitating the absorption of lipids; and conversely, we may consider useful for therapeutic purposes those substances which inhibit this absorption or modify their synthesis, such as phytosterol and phenylethylacetic acid.

The lipid theory of atherosclerosis does not provide an easy explanation for the origin of some anatomical alterations which, even in Virchow's day, had drawn the attention and stimulated the critic of many scholars.

We intend to refer to those alterations of the subintimal strata of the large and medium-sized arteries, where the main initial lesion takes the shape of a swelling of granulous substance, probably of mucoid nature, which imbibes the subintimal strata of the wall itself. In these spots, which seem to precede the formation of an atheromatous patch, only later on, lipids are deposited with a mechanism of filtration or altered

imbibition still under discussion but which seems better able than other theories to explain the deposit of blood lipids in the primitively altered wall. The primitive nature of the essentially mesenchymal lesion, with following lipid infiltration, was recently supported by many authors (21–38). Current opinion, however, favours the lipid theory without discussing or considering other possibilities. Whatever the reason for the tendency of lipids to localize on particular spots of the arterial wall in the shape of clearly limited patches, preferably on the medium-sized and larger arteries and specially the coronary arteries, local factors must be involved, otherwise the large lipid molecules, which are homogenously dissolved in the plasma, would be evenly distributed over the arterial wall forming a diffuse veil and not clearly limited patches on certain sites. If we admit, however, that on a given spot of the wall there arises a primitive alteration of the tissue favouring the permeability or the imbibition or the fixation of circulating lipoproteins, than it is easier to understand the pathogenesis of the atheromatous patches.

We may also presume that the filtration of lipid molecules through the arterial wall is normally easier in small capillary vessels and arterioles, and more difficult in vessels with media and well-developed inner elastic membrane (39). Mesenchymal tissue alterations of these strata and the subintimal tissue could reduce and stop, in clearly limited regions, the filtration of lipids and especially of the large lipoprotein molecules. At any rate, the alterations of the binding substance seem to precede the lipid lesions. Besides altering the permeability, they may increase hydrophilia and imbibition, and facilitate the fixation of lipids in regions showing such modifications. Nor can we otherwise explain why lipids are deposited like calcium, at some stages of life and in pathological conditions, in so-called bradytrophic tissues (vascular tissue, articular cartilage, cornea, tendons), the chemical composition of which shows a close analogy. As far as calcium is concerned, it has already been proved with exact chemical experiments that precipitation in these tissues takes place through a mechanism of ion exchange between chondroitin sulfuric acid in various stages of depolymerisation and blood cations, among which calcium occurs (40, 41).

A similar mechanism has been proved physiologically in the calcification of bone cartilage (42). From the chemical point of view, we may point out that these tissues are formed mainly by chondroitin sulfuric acid which determines its structural and functional characteristics, while hyaluronic acid is absent or present only in extremely small quantities (43).

Even the supposed hyaluronidase activity of the testicular extract on

the chromototropic substance of the aorta or the permeability of the vessel to lipids is not due to hyaluronidase itself, but to the associated chondroitinase activity of testicular hyaluronidase, not present in the bacterial hyaluronidase which actually does not modify the chromotropic substance of the vessels (43).

This observation confirms chemical findings which exclude the participation of hyaluronic acid in the composition of vascular tissue. The analogy between calcium and lipid deposits seems convincing enough to consider a physicochemical relation between the derivatives of chondroiting sulfuric acid and certain lipids, as happens with calcium. Recent anatomo-pathological research suggested the possibility that the alterations of the mesenchyme of the subintimal strata, with the accumulation of more or less depolymerised or denaturated mucoids, may favour the fixation of both calcium and lipids. *Buck* (44) recently studied the behaviour of polysaccharides regarding metachromasia and PAS-positivity in tissues more liable to lipid infiltration. He considers that there is no relation between the intensity of metachromasia in the tissue and that of the lipid deposits. But when we carefully observe the results submitted by this author, we may note a close correlation between intensity of PAS-reaction and lipid localization.

In this connection we recall that the metachromasia decrease of the initial atheromatous lesion is probably due to depolymerisation of tissue mucopolysaccharides.

The frequency of gerontoxon in atherosclerosis and in old age, as well as the other lipid infiltrations in organs with analogous structure, offer the clinical and anatomo-pathological confirmation of this point of view. Some authoritative German authors of *Roessle's* school have shown that lipid infiltration of articular cartilage in atherosclerosis and in old age proceeds parallel to lipid articular alterations (45). *Greppi* (46) believes that a common metabolic defect exists in atherosclerosis and arthrosis, namely a congenital defect, or an acquired primitive or secondary defect of the ground substance which we may vaguely define as "hypomesenchymosis". This alteration of the biochemistry of active mesenchymes might precede or condition the lipid metabolism itself, through a deficient liberation of heparinoid substances; these are known to be produced by the connective tissue and especially by mast cells. The importance of heparin as an activator of clearing factors and regulator of lipid metabolism, especially in atherosclerosis, has been the subject of many studies to which we also contributed (47). While we do not want to underestimate the importance of the lipoprotein alteration in the pathogenesis of the atheromatous patch, we nevertheless believe that this possible metabolic

alteration should be considered. It may be hard to find evidence for it at present but further research should be undertaken. To admit the possibility that in atherosclerosis, as well as in arthrosis, there exists a defect in tissue biochemistry and that this defect precedes and favours the localization of the lipids, means that the research on atherosclerosis must shift from alterations of the lipid metabolism to that of mesenchymal tissues. It also means the abandoning of a purely humoral pathogenetic interpretation, as has been prevailing in recent years after the research on cholesterolemia and the lipoprotein situation in blood. We must not, however, underrate the importance of cholesterol and of lipoprotein modifications, which are probably directly responsible for the lipid component in the atheromatous patch, as recent studies with radioactive isotopes seem to prove. Nor must we forget the importance of diet and of the increase of circulating lipids which at least with some patients could lead to the tissue alterations, since the tissue no longer has the metabolic capacity of coping with the excess of lipids put into circulation. We have seen how a lipid diet, through the hyperlipemia it causes, may lead to a decrease of heparin activity and tolerance, as well as of the quantity of circulating heparinoid substances (19, 20). It may be suggested that the lipids themselves, in time, indirectly lead to that exhaustion of the mechanism regulating the lipid transport which we would connect with the function of the connective tissue.

In other words, if an alteration in the freeing of heparin and in the formation of the clearing factor is considered to be responsable for the metabolic block of large lipoprotein molecules, it is also possible that a high and constant lipid supply leads to the exhaustion of the capacity of forming heparinoid substances or the clearing factor, in the same way as another exogenous or endogenous toxicosis blocks or alters certain highly specialized functions of the mesenchymal tissues.

The histochemical modifications following a depolymerisation of tissue mucoids may again facilitate the penetration or fixation of these lipids in the tissues. This would mean a single interpretation for the humoral and for the tissue theory.

We must therefore consider whether there do not exist other factors (probably tissue factors) which prevail in atherogenesis, and whether the alteration of the lipid metabolism is not an associated, but by no means indispensable factor. Finally we have the problem of arteriosclerosis, where the lipid metabolism does not seem to be involved, while vascular alterations cannot be dissociated from those noted in atheroma. The possibility that the atheromatous affection shares with arteriosclerosis the primitive alterations of the mesenchymal tissue

where plasma lipids are deposited, especially when their rate in the blood is high, makes it possible to connect the anatomo-pathological and clinical approach of these two pathological conditions (their differentiation is probably due to the alteration of lipoproteins secondarily localized on the arterial wall, thereby starting that tissue chain reaction which leads to the formation of the atheromatous lesion). We see in the atheromatous disease the lipid complication of the initial alterations of arteriosclerosis. The arteriosclerosis of the old patient would be merely the sclerotic evolution of the mesenchymal alteration of the preceding years, with decrease of the associated lipid component and the accompanying humoral involvements. The significance of lipoproteins may have brought about a new progress in the physicochemical knowledge of the structure and the transport of circulating lipids and started important research from the technical and theoretical point of view; but in our view it did not actually shift the problem of atherogenesis from what was already known about cholesterol. It was not the step from cholesterol to lipoproteins which marked a real progress in recent research, but the discovery of a substance, namely heparin, which directly and indirectly interferes with a tissue factor in the formation of the clearing factor which, by a mechanism of the enzyme type, leads to the return to normal of the altered lipoprotein metabolism.

It is this substance, then, which may be considered as a hormone of the mesenchymal tissue: that is the new discovery of recent years. All causes which may influence the production of this activator and the formation of the clearing factor may therefore indirectly influence the lipid metabolism as well. Hence the necessity of examining whether there actually exists in atherosclerosis a decreased formation of heparinoid substances or clearing factor, which would indicate a possibly diminished mesenchymal activity.

This may not be the only alteration of mesenchymal tissue; there may exist other metabolic alterations, especially of mucopolysaccharides, of which we may gain an indirect evaluation by the study of blood polysaccharides (47) .These polysaccharides are known to be directly derived from the liberation and depolymerisation of the more complex mucopolysaccharide molecules of the tissues. We tried to find in the blood the main polysaccharide groups which may be determined with easy techniques suitable for a research on a vast number of people. We used the method of anthrone, of carbazol, and of orcinol (48) in order to determine the protein sugar, (i.e. all the polysaccharides bound to serum protein precipitable with alcohol). Separately we determined glucosamine with Elson's and Morgan's method, as modified by *Schloss* (49). Although this

substance is part of the precipitable glycoprotein molecule with serum protein, it is not determined with the methods mentioned but separately after acid hydrolysis of the glycoprotein complex.

Winzler's mucoproteins (50) were determined with that author's technique, both as polysaccharides and proteins. The method of the total polysaccharides seemed the most valuable to us, since it gave the most constant results, especially with anthrone and orcinol. In contrast, the glucosamine method is influenced by the presence of amino acids and of polypeptide derivatives of protein hydrolysis and therefore is not so reliable as was thought. We believe that other, more recent, methods (51, 52) may give us more exact results. In our cases the glucosamine determination showed variations with regard to normal which were very much like those observed for total polysaccharides: which would mean that the polysaccharides determined with the anthrone method are in constant relation with glucosamine. *Winzler's* method (50) also showed us minor variations, but in the same direction as those observed when determining the total protein sugars, so that for brevity we only refer to what we observed with the help of the latter technique. In preceding studies, both on human and experimental atherosclerosis, we had observed an increase in these polysaccharides and noted an opposite course to that of heparinoid substances (53, 54, 55).

Even in the experimental atherosclerosis of the rabbit, from cholesterol, we observed a considerable increase in protein sugars after the cholesterolemia increase, at a time when the appearance of the first intimal lesions could be noted (54, 55). An experiment repeated later in collaboration with *Mininni* on a larger number of rabbits confirmed these findings (Fig. 3). In this case, of course, the high polysaccharide contents of the serum is certainly secondary to the alterations of the lipid metabolism. It may be that the altered lipid metabolism exercises an influence on the metabolism of mesenchymal tissues, from which blood polysaccharides seem to be derived, while in the light of our experience it seems scarcely probable that protein sugars derive only from the vascular alteration secondary to lipid deposits. In this connection we recall that the polysaccharide increase, both as mucoprotein and total protein sugars, was described and also studied by us in many vascular diseases, especially diabetes. At least in the latter disease, the increase of these polysaccharides seems to be indirectly connected with the glucose metabolism. The synthesis of many tissue polysaccharides, such as glucosamine, glycuronic acid, heparin, seems to be closely bound to the glucose metabolism. The synthesis of chondroitin sulfuric acid and of glycuronic acid in the skin appears to be reduced in diabetes, while an

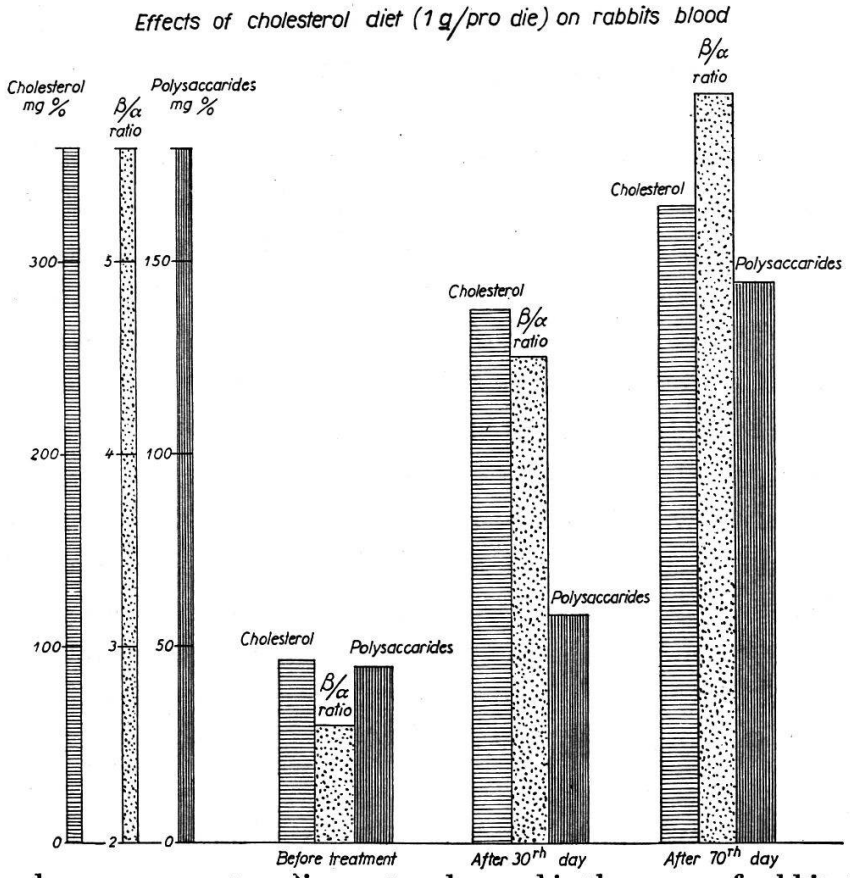

Fig. 3. The columns represent medium rates observed in the serum of rabbits before and after 30–60 days of cholesterol (1 g pro day) treatment.
Relation between polysaccarides before and after 60 days treatment $t = 9{,}08$ $P < 0{,}01$.

increase in pathological, partially depolymerised, metachromatic or PAS-positive polysaccharides is found in the connective tissue in many inflammatory diseases, such as articular rheumatism, primary chronic polyarthritis, lupus erythematosus, myxoedema, and in pregnancy, as well as after administration of oestrogen, androgen, desoxycorticosterone, anterior pituitary extract (56).

These alterations of the basic substance are found chiefly in those tissues which have a limited vascularization and the metabolism of which is considered bradytrophic and is characterised by an increase in glycolysis. These bradytrophic tissues, such as cartilage, synovia, renal medulla, intestinal mucosa, aorta, have the typical capacity, exactly like the neoplastic, embryonal, inflamed or anoxemic tissues, to produce acid mucopolysaccharides, as well as to show an increased glycolysis (56).

These alterations of tissue metabolism may of course be influenced by all those mechanisms related to glycolysis in the connective tissue: modification of pH, of electrolytes, vitamin (B, C, pyridoxine) and hormone influences. The problem concerns not only atherosclerosis, but involves the pathology of the mesenchyme, from the formation of hyaline amyloid, para-amyloid substance to fibrinoid necrosis.

The increase in polysaccharides which we observed in atherosclerotic patients shows a directly opposite course to that of heparinoid substance shown in Fig. 2, in the same group of patients. In recent research we not only carried out the routine determinations (prothrombine activity, heparin activity, antithrombine activity, cholesterolemia, protein and lipoprotein state) but compared, in a group of normal cases and cases of athero and arteriosclerosis selected as in our preceding studies, directly the values of total polysaccharides and those of matachromatic and heparinoid substances. By observing directly the results thus obtained, we noted that protein sugars increase progressively from youth over intermediate age to senility even in clinically normal persons. Similar results in senility were also obtained (57). In the atheromatous patient the serum polysaccharides are much higher than in apparently normal people. If instead we look at the chart of heparinoid substances determined in the same subjects, we observe an exactly opposite course. The rates are high in youth and decrease with age, and especially in senility, even in apparently normal people, but especially in athero- and arteriosclerotic patients. In spite of the marked oscillations which the two methods imply, and the limited possibility of distinguishing really normal individuals from pathological ones, we could determine an inverse correlation between the two phenomena, although of limited significance (Fig. 4). With a decrease of metachromatic or heparinoid substances, perhaps due to a reduced production, we note an increase in protein-bound polysaccharides which express an increased tissue catabolism with depolymerization of connectival mucoids and following liberation in the blood. This phenomenon of increased liberation of some types of polysaccharides and decreased formation of the more complex heparinoid molecules may be explained by a primitive metabolic alteration of mesenchymal tissue regulating the formation of these substances (e.g. anaerobic glycolysis). It may be objected that this type of response is common to all diseases which primarily or secondarily concern these tissues. If we observe the main inflammatory or neoplastic diseases, and even diabetic vascular diseases, we find in the blood a considerable quantity of these protein sugars, but at the same time we note an increase and not a decrease in heparinoid substances. Therefore atherosclerosis, as well as arteriosclerosis, is distinguished from other forms of disease which show a marked active involvement of the connective tissues. This humoral differentiation is probably an indirect sign of a different type of tissue alteration, possibly of an involvement of connective tissue with different polysaccharide structure.

As a matter of fact, bradytrophic tissues are essentially built up by

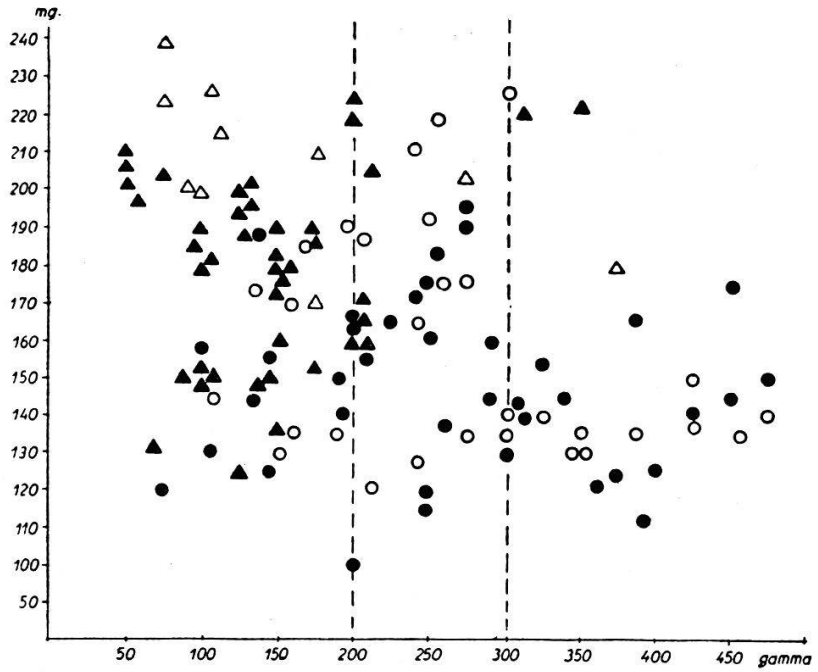

Fig. 4. Correlation between heparinoid substances and protein polysaccarides in 123 normal and atherosclerotic subjects.
Inverse correlation with $r = 0.41$, $t = 4.95$ $P < 0.01$.

chondroitin sulfuric acid and not by hyaluronic acid which prevails in phlogosis and malignant neoplasia.

Recently, to confirm the importance of the connective tissue on the factors which regulate the linkages and lipid metabolism, we have determined the effect of heparin on clearing factor activity "in vivo" in the aged.

While it was already known that there is a slight reduction of the activity of the clearing factor in atherosclerotic subjects, it was not yet known how this activity proceeded at different ages and especially during old age (Fig 5).

In the following figure, the ordinates indicate the time in minutes which is needed to reach a 50% clearing effect of the original optical density of the plasma taken half an hour after heparin injection (50 mg i.v.). To the plasma has been added a standard mixture of homogeneous lipids. On the abscissae is shown the age of the analysed subjects (58).

It may be noted that, to reach the same clearing effect, young people need a very short time while old ones need a much longer one.

As we have found (58), the time observed in old age is higher than in youth and middle age. The fault in the production of the clearing effect is higher in old age than in the middle-aged atherosclerotic subjects, although lipid alterations prevail in the latter and are of no account in senility and in arteriosclerosis.

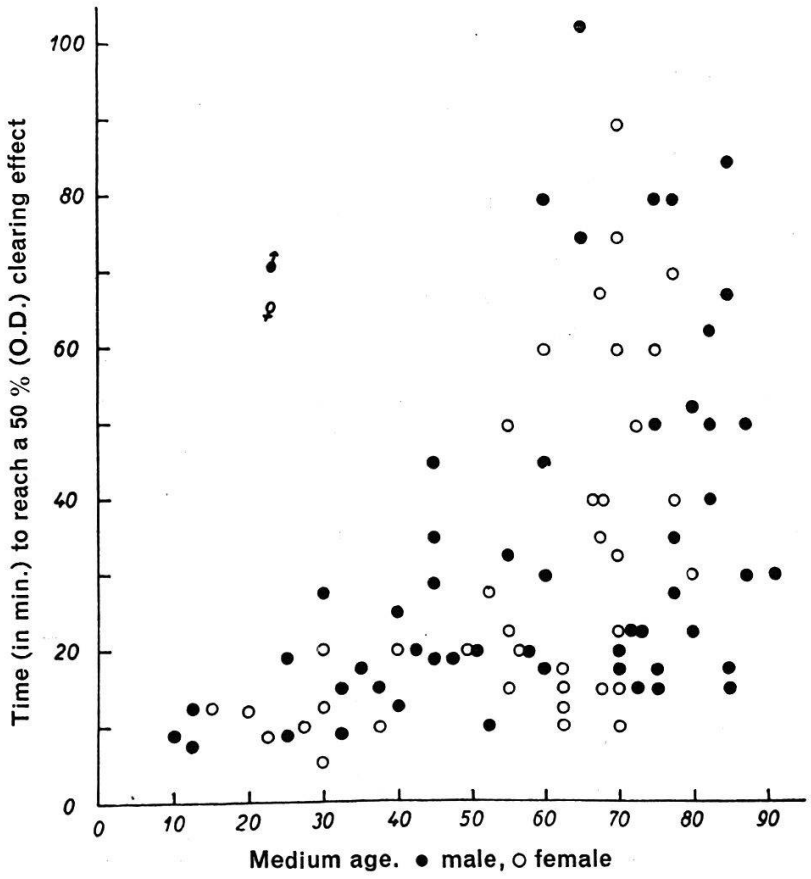

Fig. 5.

At the moment we cannot say which is the main mechanism concerned with the reduction of the clearing effect by heparin. Is it the injected heparin that acts less in elderly subjects and is not able to release the tissue factors; or are there inhibitor substances on heparin or clearing factor activity, among which some blood polysaccharides could be taken into consideration?

It can be suggested that in old age there is either a rise, not only quantitative but also, in all probability, peculiar, of these blood polysaccharides which indicates the depolymerization of tissues' mucopolysaccharides.

The abnormal increase of C reactive protein in the aged is probably an immunochemical sign of the presence of pathological glycoproteins (59).

It can be concluded that in old age blood polysaccharides increase not only quantitatively but they have also a chemical structure such as to give specific immune reactions.

It is possible that humoral alterations are not the «primum movens» in the pathogenesis of atherosclerosis, but rather the expression of a deficit in the normal production of mucoid substances, of which heparin is at present the only one with which an anti-atherogenetic activity is noted.

While diminished production of heparin by the mast cells can explain the abnormal lipoprotein distribution, the altered mucopolysaccharide

situation in the organs composed mainly of connective tissue (large vessels, tendons, cornea, cartilage) could provide a reason for the prevalent localization of lipids in these organs, not only through a defect in filtration, but through a factor of chemicophysical localization linked with the alteration in mucopolysaccharide metabolism.

Conversely, lipemia itself, either spontaneous or induced, may with the passing of years be one of the causes of this progressive functional exhaustion.

Summary

The authors have studied the situation of the lipoproteins at different ages in the two sexes both in atherosclerosis and in arteriosclerosis, and they consider that the β/α lipoproteins ratio is the most simple and indicative practical test for lipoprotein disturbances, although this is not yet a reliable test from the point of view of individual diagnosis.

The quantity of metachromatic substances (heparinoides) of the blood plasma is decreased and it is possible to show that there is an inverse proportion between this decrease and the raise of the β/α ratio. In advanced old age, this correlation between the lipoproteins and the metachromatic substances is no longer found, since there is a progressive diminution both of the plasma concentration of the latter and of the β/α ratio even in individuals who are clearly sclerotic. Parallel with the determination of the quantity of metachromatic substances, the quantity of protein sugars, glucosamine and mucoproteins of *Winzler* has been studied in old age, in arteriosclerosis, in atherosclerosis, and in experimental atheromasia of the rabbit.

The results showed above all an increase in protein sugars, particularly evident in experimental atheromasia, which is accompanied by a decrease in metachromatic substances.

These alterations seem to be the result of alterations in the biochemistry of the tissues (depolymerisation of chondroitin sulphuric acid of the vascular walls).

The alteration of the glycoproteins of the plasma may be demonstrated also in the increase which occurs in old age of protein C (C reactive protein) and of α_2-globulins.

The activity of a "clearing factor" has been discovered to be considerably more diminished in senility than in atherosclerosis of middle age.

These facts on the alteration of the plasma glycoproteins, the metachromatic substances, the «clearing factor», the protein C and the α_2 globulins suggest the possibility of an alteration in metabolism of the fundamental substance in the tissues derived from mesenchyme, parti-

cularly of the metabolism of chondroitin sulphuric acid of the vascular walls, whether it be of atherogenic origin or more especially of senile sclerotic origin.

Zusammenfassung

Die Autoren haben die Lipoproteidverhältnisse in den verschiedenen Altersstadien beider Geschlechter, sowohl bei athero- als auch bei arteriosklerotischen Patienten, untersucht und halten den β/α-Lipoproteid-Quotienten für den einfachsten und deutlichsten praktischen Test der Lipoproteidstörungen, obschon er für individuell diagnostische Zwecke noch nicht brauchbar ist.

Die Menge der metachromatischen Stoffe (Heparinoide) des Blutplasma ist vermindert, und man kann zeigen, daß zwischen dieser Verminderung und der Zunahme des β/α-Quotienten ein umgekehrtes Verhältnis besteht.

In stark vorgerücktem Alter findet man diese Korrelation zwischen den Lipoproteiden und den metachromatischen Substanzen nicht mehr, da man eine fortschreitende Verminderung sowohl der plasmatischen Konzentration dieser letzteren als auch eine Verminderung des β/α-Lipoproteid-Quotienten selbst bei ausgesprochen sklerotischen Individuen findet. Parallel zur Mengenbestimmung der metachromatischen Substanzen wurden die Mengen der Glukoproteide, der Glukosamine und der Mucoproteide von *Winzler* bei alten Leuten, bei Arterio- und Atherosklerotikern sowie bei der experimentellen Atheromatose des Kaninchens untersucht.

Man hat vor allem eine Vermehrung der Glukoproteide feststellen können, die sich speziell bei der experimentellen Atheromatose als evident erwies und von einer Verminderung der metachromatischen Stoffe begleitet war.

Diese Veränderungen scheinen die Folge histochemischer Alterationen zu sein (Depolimerysation der Chondroitinschwefelsäure der Gefäßwände).

Die Veränderung der Plasma-Glukoproteide kann außerdem auch an der Zunahme der C-Proteine (C-reaktive Proteine) und der α_2-Globuline, welche besonders im Alter auftreten, gezeigt werden.

Die Tätigkeit des «clearing-factor» erwies sich im Greisenalter als stärker vermindert als in Fällen von Atherosklerose des reifen Alters. Diese Daten über die Veränderungen der Plasma-Glukoproteide, der metachromatischen Stoffe, des «clearing-factor» des Protein C und der α_2-Globuline lassen die Möglichkeit in Erwägung ziehen, daß eine Veränderung des Stoffwechsels der Grundsubstanz der mesenchymalen

Gewebe, im besonderen des Chondroitinschwefelsäure-Stoffwechsels der Gefäßwände, am Beginn der Atherogenese stehe und in vermehrtem Maße am Beginn der senilen Sklerose.

Résumé

Les auteurs, qui ont étudié la situation lipoprotéique aux différents âges dans les deux sexes, aussi bien dans l'athérosclérose que dans l'artériosclérose, jugent que le rapport β/α-lipoprotéines est le test pratique le plus simple comme indicateur du trouble lipoprotéique, quoique celui-ci ne soit pas encore un test utilisable au point de vue du diagnostic individuel.

La quantité des substances métachromasiques (héparinoïdes) du plasma sanguin est abaissée et on peut démontrer qu'il y a un rapport inverse entre cet abaissement et l'augmentation du rapport β/α. Dans un âge très avancé, on ne retrouve plus cette corrélation entre les lipoprotéines et les substances métachromasiques, puisqu'on observe une diminution progressive, soit de la concentration plasmatique de ces dernières, soit du rapport β/α-lipoprotéique, même chez des individus franchement sclérotiques. Parallèlement à la détermination de la quantité des substances métachromasiques, les quantités du sucre protéique, de la glucosamine et des mucoprotéines de *Winzler* ont été étudiées dans la vieillesse, dans l'artério- et l'athérosclérose, ainsi que dans l'athéromasie expérimentale du lapin.

On a retrouvé surtout une augmentation du sucre protéique particulièrement évidente dans l'athéromasie expérimentale, qui s'accompagne d'une diminution des substances métachromasiques.

Ces altérations paraissent faire suite aux altérations du biochimisme des tissus (dépolymérisation de l'acide chondroïtinsulfurique des parois vasculaires).

L'altération des glycoprotéides plasmatiques peut être encore démontrée par l'augmentation – qui apparaît surtout dans la vieillesse – de la protéine C (C reactive protein) et des α_2-globulines.

L'activité du «clearing factor» a été retrouvée sensiblement diminuée, plutôt dans la sénilité que dans l'athérosclérose de l'âge mûr.

Ces données sur les altérations des glycoprotéides plasmatiques, des substances métachromasiques, du «clearing factor», de la protéine C et des α_2-globulines font envisager la possibilité qu'une altération du métabolisme de la substance fondamentale des tissus dérivés du mésenchyme, particulièrement du métabolisme de l'acide chondroïtinsulfurique des parois vasculaires, soit à l'origine de l'athérogénèse et plus encore de la sclérose sénile.

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