Employing the present technique, the fusaric acid content of the dialysed culture filtrate of *F. vasinfectum* Atk. (grown in Richard's medium in Haffkine flasks – 1·5 l medium/4 l flasks for 8 weeks) was found to be 44·6 mg/l. The dialyzed culture filtrate was concentrated to a small volume under reduced pressure prior to spotting. The fusaric acid content was estimated as indicated earlier. The concentrate was then diluted to 75% and 50% (with distilled water) and spotted again. Pure fusaric acid was added to make up the difference in each case and the total fusaric acid content estimated for recovery. The recoveries obtained at the two levels were 95·5% and 99%, respectively indicating that the method could be employed with advantage for the assay of this toxin.

Rf values of copper and copper-fusaric acid complex in different solvent mixtures.

Solvent mixtures	Rf values	
	Copper	Complex
1. *N*-butanol-HAc-water (4:1:5) .	0·494	0·273
2. *N*-butanol-HAc-water (3·5:1·5:5)	0·666	0·617
3. *N*-butanol-formic acid-water (5:3·5:5)	0·157	0·088
4. Isoamyl alcohol-HAc-water (4:1:5)	0·230	0·090
5. Isopropanol-formic acid-water (4:1:5)	0·460	0·400
6. Aq. Dioxan (1:1)	0·790	0·630
7. Aq.phenol	0·110	Not formed
8. 400 ml of aq. butanol containing 5 g 8-quinolinol to which was added 40 ml glacial acetic acid warmed to solution	0·460	0·180
9. Incorporation of 0·5 g EDTA* in 100 ml aq. butanol	0·100	Not formed
10. Impregnating the paper with 10% alumina prior to irrigation with *N*-butanol-HAC-water (4:1:5)	0·230	Not formed
11. Aq. Collidine	0·182	0·263
12. Aq. Lutidine	0·784	0·299

* Ethylene diamine tetracetate.

The Table gives the Rf values of copper and Cu-fusaric acid complex in the 12 solvent mixtures indicated therein. It may be added that the method could be employed only in the absence of interfering metabolites which would either alter the Rf value of the complex or form chelates having close Rf values. In such cases it would be necessary to use different solvents to eliminate interference and use the corresponding standard curves.

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Zusammenfassung

Es werden der chromatographische Nachweis und die Bestimmung der Fusarinsäure als Kupferchelat mittels der Rundfiltertechnik beschrieben. Die durchschnittliche Breite des Chelatstreifens steht in einem linearen Verhältnis zu dem Logarithmus der Konzentration von Fusarinsäure. In einem Kulturfiltrat von *F. vasinfectum* Atk. war ein Zusatz von reiner Fusarinsäure quantitativ nachweisbar. Rf-Werte von Cu und Cu-Fusarinsäure-Komplexen in 12 Lösungsmitteln werden angegeben.

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Carbohydrates, Collagen and Elastin of the Normal Aortic Wall and Arteriosclerotic Hyaline Plaques

Reports in the literature on the determination of the carbohydrates and of the collagen and elastin content in the normal aorta and their possible modifications in the course of arteriosclerosis, are relatively few. However studies carried out by means of metachromatic stain reactions indicate that the elastic tissue fibers of the aorta are bathed in a matrix of 'ground substance' constituted mainly of sulphated acid mucopolysaccharides, and that the alterations of this mucinous substance could be followed by modifications of the content in collagenous and elastic fibres of the aorta, which may be of fundamental importance in the evolution of arteriosclerosis[1].

Therefore, in the present research the chemical composition of hyaline plaques from arteriosclerotic aortae was investigated and compared with that of a normal aortic wall.

The samples were severed in three fractions, labelled F. I.–F. II.–F. III.–, according to NEUMAN and LOGAN[2]. We have analyzed samples of 9 different normal aortic walls of men aged from 20 to 40 years and samples collected from hyaline plaques of 9 different cases of arteriosclerosis; the amount of tissue, from each case, which was necessary for chemical analysis, was about 1 g wet weight.

The first fraction contains mainly collagen, the second fraction contains several heterogenous proteins, and the third one contains elastin. The nitrogen was determined in each fraction by Nesslerization[3]; in addition, the hydroxyproline content in F.I., in order to define its true collagen quota, was determined by the method of TROLL and CANNAN[4]. The carbohydrates were determined by the methods described by DISCHE[5]. The presence of the SO_4^- groups was investigated qualitatively by adding $BaCl_2$ 1% to the samples, hydrolized in 5 *N* HCl, dried and made up to ml 1 with water, in order to precipitate $BaSO_4$.

[1] J. F. RINEHART, in *Connective tissue in health and disease* (Munksgaard, Copenhagen 1954), p. 239.
[2] E. E. NEUMAN and M. A. LOGAN, J. biol. Chem. *186*, 549 (1950).
[3] W. W. UMBREIT, R. H. BURRIS, and J. F. STAUFFER, *Manometric techniques and tissue metabolism* (Burgess Pub., Minneapolis 1949).
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[5] Z. DISCHE in *Methods of Biochemical Analysis* (Interscience Pub. Inc., New York 1955), Vol. II, p. 313.

		Normal aorta	Pathologic aorta	t	P
Protein . } Nitrogen }	F. I	49 ± 2	53 ± 4	0·918	> 0·05
Collagen } Nitrogen }	F. I	18 ± 1,3	25·6 ± 3·1	2·478	< 0·05
Protein . } Nitrogen }	F. II	20 ± 2	29 ± 5	1·738	> 0·05
Protein . } Nitrogen }	F. III	31 ± 3	18 ± 3	3·401	< 0·01
Hexoses	F. I	1·357 ± 0·033	2·464 ± 0·163	2·327	< 0·05
	F. II	traces	0	–	–
Methyl-Pentoses	F. I	0·987 ± 0·400	0·840 ± 0·276	0·318	> 0·05
	F. II	0·351 ± 0·126	0·380 ± 0·220	0·120	> 0·05
Hexosoamines	F. I	1·173 ± 0·293	0·260 ± 0·136	11·72	< 0·01
	F. II	traces	0	–	–
Hexuronic Acids	F. I	1·122 ± 0·170	0	–	–
	F. II	0	0	–	–
SO^{--}_4	Whole sample	+ + +	+ –	–	–

The Protein and Collagen Nitrogen of the single fractions is given as a percentage of the total Nitrogen of the samples.

All the other values are expressed as g per 100 g of proteins of the corresponding fraction.

The presence or the absence of the SO^{--}_4 groups, and their approximate amount is expressed by means of the signs: + or –.

All values are the mean ± S.E.M. of 9 determinations on different samples of normal and arteriosclerotic aortae; the 't of Student' and the value of probability are given in the last two columns.

The results of our analytical determinations, together with their statistical analysis, are reported in the Table.

The above table shows that the hexose content of the F.I. in the arteriosclerotic plaques increases in a significant way as compared with the normal aortic wall, while the methylated sugars remain unchanged. Hexosoamines and hexuronic acids are contained in the normal aortic wall in an almost equimolecular ratio; in the hyaline plaques the former decrease in a highly significant way and the latter completely disappear. The carbohydrates found in the second fraction do not assume any particular interest and are more likely due to an occasional contamination during the fractionation procedure.

The nitrogen and hydroxyproline determinations in the three aortic fractions show that there is a decrease of the normal elastic tissue of the aorta, and, at the same time, an increase of the collagenous proteins at the site of the developing hyaline plaques. The increase in collagen, together with the increase of hexoses in the arteriosclerotic aorta, could suggest the presence in the plaques of a certain amount of those polysaccharides typically bound to the collagenous and reticular fibers, as reported by SCHMITT, GROSS, and HIGHBERGER[6] and by WINDRUM, KENT, and ESTOE[7], which are almost exclusively composed of hexoses. This increase in hexoses, which we[8] have observed also in the silicotic hyaline masses and in the perisplenic hyaline synechiae, seems to be a feature of the hyalinization.

The hexoso-amines and the hexuronic acids, contained in the normal aorta together with the SO^{--}_4 groups, confirm the presence in it of sulphated acid mucopolysaccharides, which are usually tested by histochemical methods or by the incorporation of ^{35}S in the metachromatic sections of the aorta[9]. The decrease of the hexoso-amines and of the SO^{--}_4 groups, and the complete disappearance of the hexuronic acids in the arteriosclerotic plaques may represent a fall in the acid mucopolysaccharidic quota of the aorta during arteriosclerotic disease. This is of considerable interest, since authors employing metachromatic staining reactions have reported that metachromatic substances disappear only in the initial stages of the disease, during the serous imbibition of the intima, but show again, to an even larger degree, at a later period, in the constituted arteriosclerotic lesions, either coming from the blood stream or being newly synthetized from the arterial wall.

However, a decrease of the hexoso-amines contained in the arteriosclerotic aorta has also been found by KYRK and DYRBYE[10], who suggest that the increased metachromasia reported during arteriosclerosis might be due to a staining alteration of the tissue mucopolysaccharides rather than to an increase of the total concentration of these compounds.

The origin of arteriosclerosis has been attributed to a depolymerization of the sulphated mucopolysaccharides, which, in the normal aorta, would maintain its elasticity and allow the filtration of the lipidic macromolecules through the arterial wall. In fact CALI[11] and SEIFTER, BAEDER, BECKFIELD, SHARMA, and ERICH[12], who believe that during spontaneous arteriosclerosis in man, there appear in the blood stream newly formed substances able to depolymerize the mucopolysaccharides

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[10] J. E. KYRK and M. DYRBYE, J. Gerontology *2*, 273 (1956).

[11] A. CALI, *Basi teoriche e sperimentali per una patogenesi enzimatica della arteriosclerosi*. 17° Quaderno della sezione perugina della SIBS (Simonelli, Perugia 1955).

[12] J. SEIFTER, D. H. BAEDER, W. J. BECKFIELD, G. P. SHARMA, and W. E. ERICH, Proc. Soc. exp. Biol. Med. *83*, 468 (1953).

(in spite of the presence of an antihyaluronidase factor recently found in the serum of older men by CASTELLANI and MARS[13]), they have been able to accelerate and to increase the entity of the experimental arteriosclerotic lesions of the rabbit, by using cholesterol and hyaluronidase to accelerate the depolymerization of the sulphated mucopolysaccharides.

Our results, which might suggest not only a depolymerization but even a disappearance of the sulphated mucopolysaccharides from the arteriosclerotic plaques, are therefore in accordance both with KYRK and DYRBYE[10] and CALI[11] and SEIFTER *et al.*[12].

So far as the increased metachromasia in the arteriosclerotic aorta is concerned, while accepting the hypothesis of KIRK and DYRBYE[12], we would suggest further that the phenomenon might be due to the presence of other substances, different from the sulphated mucopolysaccharides, and particularly of the hexoses, which are significantly increased in the hyaline arteriosclerotic plaques.

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Riassunto

È stata eseguita l'analisi chimica dei carboidrati, dei gruppi solforati, del collagene e dell'elastina contenuti nella parete aortica normale e nelle placche arteriosclerotiche jaline. L'analisi dei carboidrati e dei gruppi SO_4^- è suggestiva per la presenza di mucopolisaccaridi acidi solforati nell'aorta normale e per una loro netta diminuzione nel corso dell'arteriosclerosi. Contemporaneamente alla diminuzione di tali polisaccaridi si nota una diminuzione di elastina ed un aumento delle proteine collageni nelle placche arteriosclerotiche jaline.

[13] A. CASTELLANI and G. MARS (personal communication).

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Haemoglobin F in *Talassemia Minor*. Amino-Acid Composition

In a previous paper[1] evidence was given of the presence of alkali-resistant Hb, in some respects similar to Hb F, in some subjects suffering from *Talassemia Minor*. In the present communication, we report the data obtained on the amino acid composition of Hb F which we have been able to crystallize from the blood of a subject affected by this disease.

Hb obtained from erythrocytes repeatedly washed in saline was crystallized by HAUROWITZ's procedure for foetal Hb[2]. We easily obtained hexahedral crystals typical for Hb F, well observable macroscopically, which after repeated washing in saturated ammonium sulfate appeared under the microscope to be perfectly homogeneous in shape and size.

After heat coagulation and washing off the ammonium sulfate, the precipitate was subjected to hydrolysis in 6 *N* HCl under reflux for 24 h. Analysis of the amino acids present in hydrolysate was performed by column chromatography on Dowex 50, X–8, according to MOORE and STEIN[3], with the slight variations previously described[4]. The determinations were made in duplicate on two different 24 h hydrolisates, using for each analysis quantities of 1·61 or 2·93 mg of protein, as calculated from quadruplicate microkjeldahl and assuming a *N* content for Hb of 16·9%[5].

In the Table the data obtained are reported expressed as g amino acid/100 g protein.

Comparing these data with those obtained in our previous analysis of Hb F crystallized from placental cord blood[6], it is evident that there is a good agreement

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The amino acid composition of 24 h hydrolysate of the alkali-resistant fraction in *Talassemia Minor*.

	Talassemia Minor				Mean	Hb F[6]	Cooley's anemia[7]
Aspartic	9·93	9·63	10·32	9·93	9·95	9·59	10·90
Theronine	6·94	7·10	5·82	6·60	6·61	6·98	6·30
Serine	6·69	6·94	5·91	6·28	6·45	6·39	5·50
Glutamic	7·81	8·20	6·96	7·29	7·56	6·81	7·85
Proline	3·01	4·32	5·49	–	4·27	4·70	4·05
Glycine	4·85	4·21	4·42	3·58	4·26	3·98	4·30
Alanine	9·02	10·57	8·04	7·92	8·88	8·52	9·65
Valine	11·01	10·63	8·20	8·67	9·62	8·34	9·35
Methionine	2·28	2·31	1·69	1·67	1·98	1·84	–
Isoleucine	1·37	2·28	1·48	1·32	1·61	1·49	1·75
Leucine	15·08	16·52	15·30	13·06	14·99	13·67	15·10
Tyrosine	3·13	2·63	2·88	–	2·88	2·68	3·15
Phenylalanine	9·93	8·82	6·99	–	8·58	8·83	7·90
Lysine	9·33	9·99	9·38	–	9·56	12·08	9·70
Histidine	7·58	7·61	7·25	–	7·48	7·04	7·25
Arginine	2·91	3·11	2·83	2·79	2·91	3·11	3·30
					107·59		