(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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Clinica del Lavoro della Università di Milano, April 14, 1957.

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

[1] D. CAVALLINI, C. DE MARCO, A. ROSSI-FANELLI, and E. SILVESTRONI, G. Biochim. *3*, 307 (1954).

[2] F. HAUROWITZ, Z. physiol. Chem. *232*, 125 (1953).

[3] S. MOORE and W. H. STEIN, J. biol. Chem. *192*, 663 (1951).

[4] A. ROSSI-FANELLI, D. CAVALLINI, and C. DE MARCO, Biochim. biophys. Acta *17*, 377 (1955). – A. ROSSI-FANELLI, D. CAVALLINI, C. DE MARCO, and F. TRASARTI, Boll. Soc. ital. Biol. sper. *31*, 328 (1955).

[5] W. A. SCHROEDER, L. M. KAY, and I. C. WELLS, J. biol. Chem. *187*, 221 (1950).

[6] A. ROSSI-FANELLI, D. CAVALLINI, C. DE MARCO, and F. TRASARTI, Boll. Soc. ital. Biol. sper. *31*, 328 (1955).

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		

between these values, the small differences being imputable to the standard error of the method. The only appreciable difference is in lysine content, and this can be ascribed to its relative instability in acid solutions[6].

Recently HUISMAN *et al.*[7] have analyzed the amino acid composition of alkali resistant Hb separated by chromatography on Amberlite IRC–50 from blood haemolysate of a patient suffering from Cooley's anemia (*Talassemia Major*). Our data are also fully comparable with those obtained by these authors, and reported in the Table for comparison. HUISMAN *et al.* come to the conclusion that Hb F and the alkali resistant fraction of Cooley's anemia are in all probability the same protein.

Our results on crystallization and amino acid composition further support the hypothesis that alkali-resistant Hb present in *Talassemia Minor* and in Cooley's anemia is identical to Hb F.

C. DE MARCO and FRANCESCA TRASARTI

Institute of Biological Chemistry, University of Rome (Italy), June 3, 1957.

Riassunto

Dal sangue di un soggetto affetto da *TalassemiaMinor* si sono ottenuti cristalli tipici di Hb F.

L'analisi della composizione in aminoacidi di tali cristalli porta a concludere che la frazione di Hb alcali-resistente presente in questa malattia e l'Hb F sono identiche.

[7] T. H. J. HUISMAN, H. K. PRINS, and P. C. VAN DER SCHAAF, Exper. *12*, 107 (1956).

A Female-sterile Mutant *(Deep Orange)* of *Drosophila melanogaster* Increasing Isoxanthopterine Content

Isoxanthopterine has been found and identified in the tissues of both normal and mutant *Drosophila melanogaster*[1]. In the mutants *white* and *brown*, it disappears from the tissues during the first few days of imaginal life[2], and it is never present at any stage of development in the mutant *rosy*[3]. In the more than 25 eye colour mutants which have been examined[4], none has increased the amount of isoxanthopterine present in the tissues.

The sex-linked recessive female-sterility factor *deep orange* (*dor*) has an effect on eye colour[5]. Eyes of both males and females are light orange in newly emerged flies and darken to a deeper orange with age. A comparison of the isoxanthopterine content of wild type (Sevelen line) and *dor* flies at four different developmental stages shows a clear difference between mutant and wild type flies in isoxanthopterine content (Tables I and II). In *dor* females, the amount of isoxanthopterine in-

[1] E. HADORN and H. K. MITCHELL, Proc. nat. Acad. Sci., Wash. *37*, 650 (1951). – S. NAWA and T. TAIRA, Proc. imp. Acad. Japan *30*. 632 (1954). – E. HADORN, Exper. *10*, 483 (1954). – H. S. FORREST and H. K. MITCHELL, J. Amer. chem. Soc. *77*, 4865 (1955). – M. VISCONTINI, E. LOESER, P. KARRER, and E. HADORN, Helv. chim. Acta *38*, 2034 (1955).

[2] E. HADORN, Exper. *10*, 483 (1954).

[3] E. HADORN and I. SCHWINCK, Nature *177*, 940 (1956); Z. Vererbungslehre *87*, 528 (1956).

[4] E. HADORN and H. K. MITCHELL, Proc. nat. Acad. Sci., Wash. *37*, 650 (1951). – E. HADORN, Exper. *10*, 483 (1954). – E. HADORN and I. SCHWINCK, Nature *177*, 940 (1956); Z. Vererbungslehre *87*, 528 (1956).

[5] D. J. MERRELL, Amer. Naturalist *81*, 399 (1947).

Table I

Differences in isoxanthopterine content of + and *dor* males at different developmental stages, expressed in percentage of + value.

Developmental stage	+♂♂*		*dor*♂♂			*t*	*p*
	n	SE	*n*	$\bar{x}$	SE		
Prepupa	26	1·41	28	63·82	2·26	12·54	< 0·001
50 h pupa	21	1·62	21	72·57	2·11	10·63	< 0·001
Emergence (bodies only)	15	1·82	16	99·31	2·21	0·19	not significant
4 day imago (bodies only)	13	1·86	14	74·50	3·59	4·01	< 0·001

* Because of variations from chromatogram to chromatogram it was not always possible to make a direct comparison of fluorescent values; therefore the mean value of the + readings on any one sheet was given a value of 100·0% and all readings converted to equivalent percentage values. The figures in the third column give the standard error of + male readings.

Table II

Differences in isoxanthopterine content of +, *dor* and *ClB/dor* females at different developmental stages, expressed in percentage of + value.

Developmental Stage	+♀♀*		*dor*♀♀			*t*	*p*	*ClB/dor*♀♀			*t*	*p*
	n	SE	*n*	$\bar{x}$	SE			*n*	$\bar{x}$	SE		
Prepupa	8	2·99	17	117·65**	3·41	4·05	0·001					
50 h pupa	12	2·63	11	165·28	9·51	6·73	0·001	11	143·91	3·30	10·48	< 0·001
Emergence (bodies only)	16	3·54	14	207·0	14·21	7·31	0·001	15	175·10	5·21	12·25	< 0·001
4 day imago (bodies only)	25	2·40	23	233·43	6·78	18·38	0·001	19	131·74	4·40	6·30	< 0·001

* As in Table I, all figures are converted to percentage of + mean value on each chromatogram, the + value being equal to 100·0%.
** At this stage, it is not possible to distinguish between *dor* and *ClB/dor* females; this measurement represents their combined values.