Free Amino Acid Patterns and other Chemical Constituents of Rabbits' Blood and Blood Vessels[1]

To date, the chemical structure of normal and diseased blood vessels of animals and man has been studied but incompletely. Some data on lipids, especially on cholesterol, and sporadic data on mucopolysaccharides, are available. The study of amino acids has been greatly neglected.

A study of blood vessels of normo- and of hypercholesteremic New Zealand white rabbits was undertaken. The question posed was the possible relationship between blood serum and various intact and altered sectors of blood vessels with regard to amino acid patterns and other chemical substances.

Tissue extract from ascending aorta and arch, thoracic aorta, abdominal aorta and the *vena cava* were prepared. Of the 25 principal amino acids 21 were found in extracts of rabbits' blood vessels but only 16 in blood serum of the same animals by circular paper chromatography. This method, although not suitable for quantitative assays, proved useful for purposes of orientation.

The pattern of free *amino acids* for the same vascular sector differed from one rabbit to another. Distribution of amino acids in the three aortic sectors from one and the same rabbit was not the same. It was impossible to correlate the amino acid spectra of different animals nor for a single animal, regardless whether one attempted to group the amino acids by their chemical characteristics or tried to follow the distribution of essential amino acids. The only amino acid derivative detected occasionally in one or another vascular segment was α-aminobutyric acid.

In rabbits with plaques in the ascending aorta the frequency of amino acids was less than in the intact descending aorta. In animals whose thoracic aorta was diseased in addition to the ascending part, the frequency of amino acids was lowered in both affected sectors. The shift paralleled the degree of disease. As the frequency of free amino acids decreased in the altered sectors it increased in the intact abdominal aorta.

Of interest was the shift of methionine in the aorta from disease of proximal sectors to intact distal parts. Glutamic acid and leucine increased parallel to tissue damage. Alanine and isoleucine were often present in diseased aorta but less frequent in normal sectors (Table I).

Total *protein* of the *vena cava* and the abdominal aorta of normal rabbits was about 30% lower than that of the other aortic sectors. The thoracic aorta had, as a rule, the highest protein content. The total protein in the ascending and thoracic sectors of hypercholesteremic rabbits was 30% higher than in the abdominal aorta and 54% higher than in the *vena cava*. The ascending and abdominal aorta had in these animals, on the average, a 25% higher *lipid* content than the thoracic aorta or the *vena cava*. Thoracic aorta *cholesterol* of normal rabbits was usually 9% lower than that of other aortic segments or *vena cava*. Cholesterol in the ascending aorta of hypercholesteremic rabbits was $2^1/_2$ times higher than in the thoracic aorta and 4 times the amount detected in the abdominal sectors (Table II).

All rabbits of the experimental series had elevated blood lipid, cholesterol and protein. All these substances were also increased in the aortic extract. However, some rabbits with extreme hyperlipemia-hypercholesteremia had less lipid and (or) cholesterol in their aorta than other rabbits with a lesser degree of blood lipid and cholesterol elevation. These substances were lowest in the abdominal aorta of animals in whom plaques were present in the

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Tab. I. Frequency of free amino acids in blood vessels and in serum of normo- and of hypercholesteremic rabbits

No.	Free aminoacids	Ascending	Thoracic	Abdominal	All Aorta	*Vena cava*	Blood serum	Ascending	Thoracic	Abdominal	All Aorta	*Vena cava*	Blood serum
1	Alanine	9.0	25.0	16.0	17.0	25.0	50.0	30	20	20	23.0	10	10
2	Arginine	18.2	0.0	25.0	14.3	37.4	50.0	30	40	40	36.7	0	60
3	Aspartic acid	0.0	0.0	16.6	5.7	12.5	10.0	30	30	20	26.8	0	10
4	Cysteine	36.4	25.0	33.3	31.4	62.5	50.0	30	40	60	43.0	30	50
5	Cystine	82.0	100.0	100.0	94.0	100.0	100.0	80	80	80	80.0	70	100
6	Ethionine	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0	9.0	0	0
7	Glutamic acid	18.2	25.0	33.3	25.6	12.5	20.0	70	70	70	70.0	80	90
8	Glutathione, red.	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0	0.0	0	0
9	Glycine	45.5	16.6	42.0	34.4	12.5	20.0	50	30	50	43.0	80	80
10	Histidine	9.0	25.0	8.4	14.3	12.5	30.0	60	30	50	47.0	40	20
11	Homocysteine	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0	0.0	0	0
12	Hydroxyproline	0.0	0.0	8.4	2.9	0.0	10.0	0	0	0	0.0	0	0
13	Isoleucine	18.2	16.6	25.0	20.0	37.4	0.0	50	50	50	50.0	20	10
14	Leucine	27.2	33.3	16.6	25.8	50.0	10.0	70	60	40	56.5	50	10
15	Lysine	36.4	33.3	42.0	37.2	50.0	50.0	50	50	40	47.0	40	20
16	Methionine	36.4	58.0	25.0	40.0	75.0	20.0	20	30	50	33.0	60	20
17	Norleucine	36.4	16.6	33.3	28.6	37.4	0.0	70	80	60	70.0	40	0
18	Phenylalanine	36.4	25.0	25.0	28.6	50.0	0.0	50	40	30	40.0	40	20
19	Proline	18.2	25.0	42.0	28.6	50.0	20.0	20	40	50	36.6	60	50
20	Serine	0.0	16.6	0.0	5.7	12.5	30.0	10	30	20	20.0	20	0
21	Thiolhistidine	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0	0.0	0	0
22	Threonine	36.4	33.3	42.0	37.2	37.4	50.0	60	90	90	80.0	50	80
23	Tryptophane	18.2	0.0	16.6	11.4	0.0	0.0	0	10	10	6.6	0	0
24	Tyrosine	45.5	16.6	25.0	28.6	25.0	0.0	20	70	60	50.0	40	40
25	Valine	9.0	8.4	33.3	17.1	0.0	10.0	40	0	30	23.0	0	0
	α-Aminobutyric acid	9.0	0.0	16.6	8.6	25.0	0.0	10	10	0	6.6	0	0

Amino acids listed alphabetically; frequency expressed in %; first six columns refer to normal rabbits, second set of six columns refer to experimental animals.

Tab. 2. Amounts of total protein, lipid and cholesterol and number of free amino acids in blood vessels and in serum of normo- and of hypercholesteremic rabbits

Per 100 ml of tissue extract or blood serum		Ascending min.	avg.	max.	Thoracic min.	avg.	max.	Abdominal min.	avg.	max.	All Aorta min.	avg.	max.	*Vena cava* min.	avg.	max.	Blood serum min.	avg.	max.
total protein	(g)	1.11	2.09	3.40	1.84	2.83	3.80	1.35	1.92	2.94	1.84	2.28	3.23	0.63	1.51	2.20	4.25	5.75	7.85
total lipid	(mg)	110	212	555	29	145	485	70	178	545	88	178	528	60	148	470	215	566	1000
total cholesterol	(mg)	1.70	3.30	5.90	1.00	3.05	6.60	1.10	3.40	11.0	1.63	3.25	6.60	1.00	3.29	5.30	33.0	62.3	84.0
No. of free aminoacids		4	5.45	7	3	5.00	8	3	6.35	8	4	5.60	8	5	6.44	8	4	5.30	6 ·
Per 100 ml of tissue extract or blood serum		**Ascending min.**	**avg.**	**max.**	**Thoracic min.**	**avg.**	**max.**	**Abdominal min.**	**avg.**	**max.**	**All Aorta min.**	**avg.**	**max.**	***Vena cava* min.**	**avg.**	**max.**	**Blood serum min.**	**avg.**	**max.**
total protein	(g)	2.96	5.60	8.70	3.11	5.12	7.90	2.20	3.70	5.55	2.91	4.86	6.80	1.06	2.34	5.65	7.55	8.89	10.4
total lipid	(mg)	242	621	985	305	457	690	222	492	800	274	523	778	90	154	200	1710	2199	3490
total cholesterol	(mg)	12.0	32.0	50.0	6.30	13.1	22.0	2.90	8.07	19.7	7.90	17.5	30.6	4.90	9.65	15.0	440	2869	5800
No. of free aminoacids		7	8.5	10	7	9	10	7	9.2	10	7	8.7	10	6	7.3	8	6	6.7	8

Minimum, average and maximum amounts of total protein (expressed in g/100 ml of tissue extract or serum), of total lipid (as mg/100 ml) and of total cholesterol (as mg/100 ml); minimum, average and maximum number of free amino acids detected in this material.

thoracic segment as well as in the ascending aorta. The reverse was found for protein and for the frequency of free amino acids. The amount of lipid and cholesterol could not be correlated with the type of amino acids recovered from blood vessels whether one compared the same sector from different rabbits or various sectors from the same animal.

The inverted relation between total protein and the number of free amino acids in normal vascular tissue should be noted and also a similar relationship between total protein and total lipid-cholesterol. Segmental differences could signify local differences in protein hydrolysis or in protein synthesis.

The lack of direct proportion between lipid and cholesterol of the diseased aorta and the degree of hyperlipemia and hypercholesteremia had not been anticipated since the chain of 'cholesterol diet–hypercholesteremia–cholesterol atherosclerosis' has been well established for the rabbit. The validity of the filtration theory for human atherosclerosis may be debatable although it has found widespread acceptance. We are reminded of anatomic structural differences of various parts of the aorta. With regional variations in the amount of endothelial, muscular, connective tissue and other elements one may expect differences in chemical composition as well.

The fact that chemical changes were present in grossly intact aortic sectors of hypercholesteremic rabbits indicates that chemical changes precede anatomical alterations.

Zusammenfassung. Die Verteilung der freien Aminosäuren, von Protein, Fett und Cholesterin im Blut und in verschiedenen Gefässen normaler und hypercholesteremischer Kaninchen wurde untersucht. Geschädigte Aortaabschnitte erhielten mehr Protein, Fett und Cholesterin, aber weniger freie Aminosäuren als normale.

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Release of Histamine by Cotton Dust Extracts from Human Lung Tissue *in vitro*

In previous communications[1,2] the hypothesis has been advanced that at least some of the symptoms of byssinosis (cotton worker's pneumoconiosis) are caused by a histamine-releasing substance present in the dust inhaled by cardroom workers in the cotton industry.

This hypothesis was based on the results of dust inhalation experiments in man and on some evidence derived from application of cotton dust extracts in animal preparations. Further evidence from animal experiments supporting such a theory has been published by ANTWEILER[3,4].

It is well known, however, that histamine release varies with the tissue and animal species under study, and these differences in actions of histamine-releasing substances are only partly explained by tissue and species differences in histamine content[5].

Our own previous experiments, as well as those made by ANTWEILER, provided only indirect evidence in favour of histamine release as a causal factor in byssinosis. It remained to be demonstrated that cotton dust contains a substance capable of releasing histamine in human lung tissue. This communication presents evidence that this is indeed the case.

An extract of cotton dust was prepared as previously described[2], except for the use of Tyrode solution instead

Tab. I. Histamine release from human lung tissue *in vitro*

Sample No.	1	2	3	4	5	6
Amount of lung tissue (g)	1.2	1.2	–	–	1.2	1.2
Tyrode solution (ml)	2	2	4	4	3	3
Cotton dust extract (ml)	1	1	1	1	–	–
Histamine in filtrate (μg)	1.4	1.4	0.1	0.1	0.6	0.7

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[5] W. D. M. PATON, Pharmacol. Rev. *9*, 269 (1957).