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			Thoracic			Abdominal			All Aorta			*Vena cava*			Blood serum		
		min.	avg.	max.	min.	avg.	max.	min.	avg.	max.	min.	avg.	max.	min.	avg.	max.	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			Thoracic			Abdominal			All Aorta			*Vena cava*			Blood serum		
		min.	avg.	max.	min.	avg.	max.	min.	avg.	max.	min.	avg.	max.	min.	avg.	max.	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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Tab. II. Histamine release from human lung tissue *in vitro*

Sample No.		1	2	3	4	5	6	7	8	9	10	11	12
Amount of lung tissue	(g)	2	2	—	—	2	2	2	2	2	2	2	2
Tyrode solution	(ml)	2	2	4	5	3	3	2	2	2	2	3	3
Cotton dust extract	(ml)	1	1	1	1	—	—	—	—	1	1	—	—
								48/80		Histamine			
								1 mg in 1 ml		10 μg in 1 ml			
Histamine in filtrate	(μg)	2.2	1.8	0.1	0.1	0.9	0.9	1.8	2.0	7.5	6.3	16.0	20.0

of saline. The sample of cotton dust was collected in a ventilator in a card-room in the same way as the dust previously used.

Specimens of macroscopically normal human lung tissue were obtained from patients operated upon for bronchial carcinoma (Table I) or pulmonary tuberculosis (Table II). The lung tissue was minced with scissors and washed once with Tyrode solution.

Aliquots of lung tissue weighing 1.2 or 2 g were suspended in 2 ml of Tyrode solution containing 1 mg of glucose per ml[6] and 1 ml of cotton dust extract added (Tables I and II, samples 1 and 2).

The incubation, which was done in a shaking incubator at 37°C, lasted for 20 min.

The suspension fluid was separated from the lung tissue particles by filtration through sintered porcelain filters. The filtrate was heated to boiling and diluted to 5 ml with Tyrode solution.

The following controls were employed: (a) The spontaneous histamine release in lung tissue during the incubation was measured (Tables I and II, samples 5 and 6). (b) The amount of histamine derived from the cotton dust extract itself was measured (Tables I and II, samples 3 and 4). (c) For comparison the histamine release caused by compound 48/80, a well known histamine-releasing drug, was studied (Table II, samples 7 and 8). (d) To get some information on the possible inactivation of some of the released histamine during the incubation, 10 μg of histamine base was added to the complete incubation mixture (Table II, samples 9 and 10). (e) To estimate the total histamine content in the aliquots of lung tissue, 2 g of tissue in 3 ml Tyrode solution were boiled immediately[7] and then filtered (Table II, samples 11 and 12).

The amount of histamine in the filtrates was measured by bioassay on the isolated guinea-pig ileum. The specificity of the gut-contracting activity was tested with an anti-histamine drug (mepyramine) in a concentration just sufficient to inhibit completely equiactive doses of histamine. The person who performed the bio-assay had no knowledge of the incubation schemes.

The results are shown in Tables I and II. In both series of experiments, the amount of histamine released in the samples containing cotton dust extract was about twice as much as the amount spontaneously released from lung tissue. The cotton dust extract itself released negligible amounts of histamine. This is in agreement with earlier observations[2].

Compound 48/80 caused release of histamine quantitatively comparable to that induced by cotton dust extract. The amount of histamine released both by cotton dust extract and by 48/80 corresponded to about 5% of the total histamine content of the lung tissue. This may be compared with the results of MONGAR and SCHILD[8], who found that 48/80 released about 13% of the histamine in guinea-pig lung.

LILJA et al.[9] have found that human lung tissue can inactivate histamine by methylation *in vitro*. It was therefore considered desirable to measure the inactivation of histamine under the present conditions (Table II, samples 9 and 10). Histamine release by cotton dust extract accounts for about 2 mg of histamine in the filtrate of these samples (cf. samples 1 and 2); it is therefore estimated that about 50% of added histamine was inactivated during the 20 min incubation. Therefore, the measured amounts of histamine released from the lung tissue in our experiments appear to be underestimates of the true values.

The results of the present investigation are considered to provide additional evidence in favour of the hypothesis that some of the symptoms of byssinosis in card-room workers are caused by the presence of a histamine-liberating substance in cotton dust. This mechanism of histamine-release does not require previous sensitization as do the mechanisms previously proposed by PRAUSNITZ[10] and by HAWORTH and MCDONALD[11].

Zusammenfassung. Baumwollextrakt, bei 37° mit zerkleinertem menschlichem Lungengewebe inkubiert, setzt daraus Histamin in ähnlichen Mengen wie Compound 48/80 frei.

Die Ergebnisse stützen die Hypothese, dass die Symptome der Byssinosis bei Baumwollspinnerei-Arbeitern mindestens teilweise durch eine Histamin-freisetzende Substanz im Baumwollstaub verursacht werden.

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