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Research on the Chemical Composition of the Vascular Wall XV. Collagen and Elastin of the Aorta Wall of Persons who Died of Vascular Complications in the Course of *diabetes mellitus*

Badania nad składem chemicznym ściany naczyniowej. XV. Kolagen i elastyn ściany tętnicy głównej u ludzi zmarłych z powodu powikłań naczyniowych w przebiegu cukrzycy

Исследования химического состава сосудистой стенки. XV. Коллаген и эластин стенки аорты у людей умерших вследствие сосудистых осложнений при сахарном диабете

Metabolic disturbances accumpanying *diabetes mellitus* are an accepted risk factor as far as atherosclerosis goes. As it can be deduced from many experiments they cause a number of morphological changes in the smooth muscle cells of the vascular wall, due to, among other things, defective lipid metabolism and the change in activity of some enzymatic systems (6, 11). Comparatively little is known how the defectiveness of carbohydrate metabolism influence the synthesis and deposition of extra-cellular compounds of the connective tissue, mainly collagen and elastin.

In our previous experimental work (9) we ascertained that short — lasting alloxan diabetes does not cause essential changes in the content of collagen and elastin of medial and internal layer of the aorta. Present investigations aimed at determining the collagen, elastin and supporting proteins content in the aorta of patients suffering from chronic *diabetes mellitus* and whose death resulted from vascular complications.

MATERIAL AND METHODS

Investigations have been carried out on 16 patients suffering from *diebetes mellitus* whose death resulted from vascular complications (myocardial infarction, cerebral haemorrhage) the autopsies were carried at the Department of Patholo-

gical Anatomy of the Medical Academy in Lublin. The age of the patients ranged between 48 and 84 ,the average being 67,4±8,4. They had suffered from metabolic *dibetes mellitus* and had been cured by a special diet and hypoglcemic drugs. In all the patients macroscopic and microscopic features of the generalized otherosclerotic process were ascertained. The control group consisted of 10 patients, age 52—82 the average being 72.3 ±8.6, who died of other causes and in whom no macroscopic atherosclerotic changes were observed.

Investigations were carried out on preparations from the descending part of the aorta taken within 12—24 hours after death. Internal and medial layers were isolated by the, Wolynsky (12) method. The method of preparating the material, the division of protein fractions in aorta wall and analytic methods which were applied are described in our prevous publication (9). The protein content in particular fractions was calculated from a determinated amount of nitrogen using the coefficient 5,55 and the amount of collagen fractions from the determinated amount of hydroxyproline multiplied by the coefficient 7,14.

The obtained results ,were calculated per 19 of moist tissue and analysed statistically according to Student's t test.

RESULTS OF INVESTIGATIONS

Results of the division and determination of soluble proteins and collagen fractions of the intima — media layers of the aorta in the investigated groups are presented in table 1. The medial and internal layer of aorta of patients suffering from *diabetes mellitus* who died of

Table 1. Soluble proteins and collagen in the medial and internal wall of the main aorta (average ±SD)

Group	Proteins mg/gwt	OH-Pro mg/gwt	Collagen mg/gwt	
	Fraction soluble in 0.45 M NaCl			
Control	26.6 ±8.0	0.20 ±0.09	1.45 ±0.72	
Investigated	20.5 ±9.0*	0.14 ±0.09*	0.98 ±0.72*	4.46
	Fraction soluble in 0.5 M CH_3COOH			
Control	5.7 ±2.3	0.11 ±0.04	0.79 ±0.29	13.74
Investigated	4.9 ±0.05	0.09 ±0.05	0.68 ±0.37	14.72
	Fraction in soluble (after gellification)			
Control	47.7 ±9.8	3.82 ±0.84	27.92 ±6.15	58.47
Investigated	45.5 ±10.7	4.79 ±1.75**	35.03 ±6.44**	78.77
	Fraction soluble in 0.1 M NaOH			
Control	11.0 ±5.1	0.30 ±0.20	2.22 ±1.50	20.15
Investigated	12.2 ±4.5	0.33 ±0.15	2.42 ±1.10	21.81
	Collagen fraction — total			
Control	91.0 ±25.2	4.43 ±1.18	32.38 ±8.66	35.58
Investigated	83.1 ±25.2	5.35 ±2.05	39.11 ±8.63	47.06

* — p 0.005.
** — p 0.025.

Table 2. Total protein, proline and hydroxyproline of the elastin fraction in the medial and internal layer of aorta (average ±SD)

Group	Protein mg/gwt	Proline		Hydroxyproline		Pro
		mg/gwt	% BC	mg/gwt	% BC	
Control	34.6 ±18.4	6.07 ±2.78	17.5	0.78 ±0.30	2.25	7.78
Investigated	45.4 ±21.8	5.49 ±2.74	12.1	0.72 ±0.32	1.58	7.62

vascular complications has revealed an essentially lower content of collagen soluble in 0.45M NaCl and a higher content of insoluble collagen in comparison to the aortas in the control group. It is also worthy of attention that the percent of hydroxyproline in the insoluble fraction is higher in relation to the total protein of this fraction. No essential differences as far as the remaining collagen fractions and the content of soluble proteins in aortas have been observed.

Table 2. presents results of determination of the total protein, proline and hydroxyproline in elastin fraction. Great individual differences in the content of the total protein have been observed, especially in the investigated group and the decrease in the content of proline and hydroxyproline calculated per 1 mg of elastin protein in aortas of the patients suffering from *diabetes mellitus* seems to be a characteristic feature.

Although the comprehensive results of the determination of the supporting protein, collagen and elastin of the medial and internal layer of the descending port of aorta in control and experimental group do not show statistically important differences, they may suggest an occurrence of the qualitative changes within the connective tissue of aorta in patients with chronic disturbances in carbohydrate metabolism. (Tab. 3).

DISCUSSION

The carried out investigations show that chronic metabolic disturbances accompanying *diabetes mellitus,* in relation to collagen of medial and internal layer of the aorta seem to us as if to intensify the changes which are characteristic for the process of physiological ageing observed in our previous publication (10).

As it is presented in Fig. 1, in comparison with the control group of the same age, the amount of insoluble collagen increases while newly — synthesized forms soluble in neutral salts and weak acids decrease at the same time.

The causes of the observed changes are difficult to explain at the present state of knowledge. Both local changes connected with presence of atherosclerotic plaques and general structural changes denoting the whole amount of collagen in connective tissue may be involved here. In the

Table 3. Protein composition of the medial and internal layer of human aorta (average ±SD)

Group	Total protein mg/gwt	Protein fractions						Collagen Elastin
		Supporting proteins		Collagen		Elastin		
		mg/gwt	%	mg/gwt	%	mg/gwt	%	
Control	125.6 ±43.6	58.62—16.54	46.7	32.38—8.66	0.94	34.58—18.44	25.8	27.5
Investigated	128.5 ±46.3	43.99—16.57	34.2	39.11—8.63	0.86	45.38—21.08	30.4	35.3

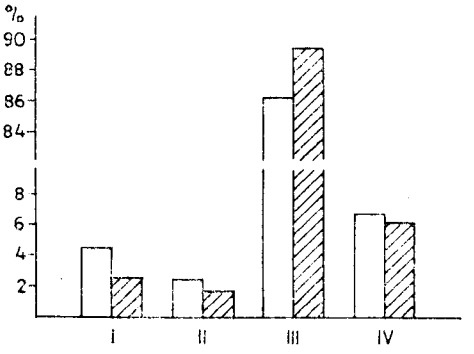

Fig. 1. Composition of collagen fraction in the intimamedia layer of the descending aorta in percent. I — collagen soluble in 0.45 M NaCl, II — collagen soluble in 0.5 M CH_3COOH, III — in soluble collagen, IV — collagen soluble in 0.1 NaOH empty spaces — control group, printed spaces — investigated group

research on the influence of insulin on the chemical composition of the vascular wall no differences in the content of collagen in the intimamedia layer have been observed (2). On the other hand it is well known that hormonal equilibrium may have an essential influence on a number of enzymatic systems engaged in the synthesis and catabolism of collagen in the connective tissue (4, 7, 8). On the basis of this fact the results of the carried out experiments may suggest that chronic metabolic disturbances accompanying *diabetes mellitus* decrease the degree of regeneration of collagen in aorta wall and perhaps as a result of increasing the number of active aldehyde groups, they enlarge the number of cross-linkages which stiffen the structure of fibrilles (1, 3).

Fig. 2 presents protein composition of the intima-media layer of the aorta in investigated groups.

In contrast to the decrease in the elastin content of aorta which is observed in the process of physiological ageing (10) an increased content of this scleroproteid has been observed in diabetic group. The relation

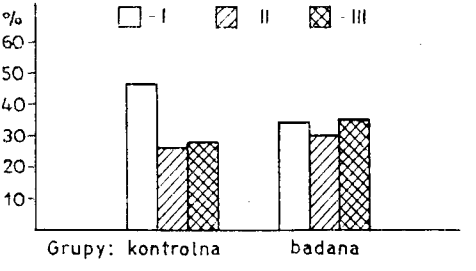

Fig. 2. Protein composition of the medial and internal layer of the main aorta. I — supporting proteins, II — collagen, III — elastin

of this phenomenon to the increased intensification of atherosclerotic changes in the investigated group may be explained by the existence of of essential qualititative changes within elastin in the aorta wall (5,13). This is also supported by a various content of proline and hydroxyproline in the elastin fraction in the control and experimental group (tab. 2), suggesting differences in the aminoacid composition in the investigated fractions. Confirmation of these assumptions requires further investigation.

CONCLUSIONS

1. In patients who died because of vascular complications in the course of *diabetes mellitus* the decrease in collagen soluble in 0,45 M NaCl and the increase in the content of insoluble collagen of the intima-media layer of aorta have been observed.

2. In comparison to the control group of the same age in the aorta of diabetic patients a higher content of the elastin fraction has been observed.

REFERENCES

1. Bailey A. J., Robins S. P., Balian G.: Nature **251**, 105—109, 1974.
2. Brosnan M. E., Sirek O. V., Sirek A., Przybylska K.: Diabetes, **22**, 297—402, 1973.
3. Deshmukh K., Nimni M. E.: Biochem. J **112**, 397—405, 1969.
4. Hanzlik J., Tomaszewski J.: Pol. Endocrin. **22**, 275—279, 1971.
5. Keeley F. W., Partridge S. M.: Atherosclerosis **19**, 287—296, 1974.
6. Kremer G. J., Niemczyk H., Knick B.: Schweiz. Med. Wschr. **97**, 735—740, 196.
7. Langer R. O., Fuller G. C.: Atherosclerosis **17**, 463—469, 1973.
8. Scott P. J.: New Zealand Med. J. **77**, 1—6, 1973.
9. Tomaszewski J., Hanzlik J., Łopatyński J., Wolański I., Grzywa M., Zawiślak H.: Pol. Arch. Med. Wewn. **50**, 793—800, 1973.
10. Tomaszewski J., Hanzlik J., Grzywa M., Zawiślak H., Stępień A.: Pol. Arch. Med. Wewn. (in press).
11. Wellmann K. F., Volk B. W.: Arch. Pathol. **90**, 206—217, 1970.
12. Wolynsky H.: Circ. Res. **26**, 507—522, 1970.
13. Yu S. Y.: Lab. Invest. **25**, 121—125, 1971.

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STRESZCZENIE

Przeprowadzono badania kolagenu, elastynu i innych białek warstwy środkowej i wewnętrznej tętnicy głównej 16 osób zmarłych z powodu powikłań naczyniowych w przebiegu cukrzycy i 10 osób grupy kontrolnej w tym samym przedziale wieku.

W grupie badanej stwierdzono istotne obniżenie frakcji kolagenu rozpuszczalnego w 0,45 M NaCl i wzrost kolagenu nierozpuszczalnego. Natomiast w przeciwieństwie do zmian obserwowanych w procesie fizjologicznego starzenia w aortach zmarłych z cukrzycą wzrastała zawartość frakcji elastynowej. Zawartość proliny i hydroksyproliny tej frakcji wskazuje na istotne różnice jakościowe w porównaniu z grupą kontrolną.

РЕЗЮМЕ

Проведены исследования коллагена, эластина и других белков среднего и внутреннего слоёв аорты у 16 человек умерших вследствие сосудистых осложнений при сахарном диабете и у 10 человек того же возраста, составлявших контрольную группу.

В обследуемой группе отмечено значительное понижение фракций растворимого коллагена в 0,45 M NaCl и повышение нерастворимого коллагена. В свою очередь, в противоположность изменениям, наблюдаемым во время физиологического процесса старения в аортах умерших больных с сахарным диабетом, повышалось содержание эластиновой фракции. Содержание пролина и гидроксипролина этой фракции указывает на значительную качественную разницу по сравнению с контрольной группой.