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# Research on the Chemical Composition of the Vascular Wall. XVI. Glycosoaminoglycans of the Aorta Wall of Persons who Died of Vascular Complications in the Course of *diabetes mellitus*

Badania nad składem chemicznym ściany naczyniowej. XVI. Glikozoaminoglikany ściany tętnicy głównej u ludzi zmarłych z powodu powikłań cukrzycy

Исследования химического состава сосудистой стенки.
XVI. Глюкозоаминогликаны аорты у людей умерших вследствие сосудистых осложнений при сахарном диабете

Vascular complications in the course of disturbances in the carbohydrate exchange seem to result in death in a majority of patients suffering from *diabetes mellitus*. As it can be seen from our previous experiments (8, 19) and the research by other authors, experimental *diabetes mellitus* is the factor which precipitates the formation of atherosclerotic changes. Glycosoaminoglycans of the connective tissue of the vascular wall are assumed to play on important role in these processes.

The aim of this report were investigations of the content of glycosoaminoglycans fractions in the aorta wall in people who had died of vascular complications in the course of *diabetes mellitus*, as compared with the control group of the same age in which no morphological changes characteristic of vascular sclerosis were observed.

## MATERIALS AND METHODS

Investigations were carried out on 16 *diabetes mellitus* patients who had died because of vascular complications and whose authopsy was made in the Department of Pathological Anatomy at the Medical Academy in Lublin. The experiment included 10 woman and 6 men age from 48 to 84, the average being 67,4±8,4. All the deceased patients had suffered from *diabetes mellitus*, for several years and had been cured by a special diet and hypoglycemic drugs. In all patients features of generalized atherosclerotic process were discovered macroscopically.

The investigations were carried out on the descending aorta. The control group consisted of preparations of the main aorta from 10 patients of the age ranging from 50 to 2, the average being 72.3±8.6, in which no sclerotic changes within aorta had been found out by the way of autopsy.

The method of preparing material for this investigation, the way of isolating and fractioning the glycosoaminoglycans of the intima-media layer is described in detail in one of our previous publications (18). As the result of the applied procedure the total content of glycosoaminoglycans and the content of the following fractions has been stated: I fraction noncomplexing with cethylopirydine chloride, II fraction of hialuronic acid, III fraction of heparane sulphate, IV fraction of chondroitine-4-sulphate, V fraction of chondroitine-6-sulphate, VI fraction of dermatane sulphate and VIII fraction of heparine. The amount of present glycosoaminoglycans was expressed in μg of uronic acid per 100 mg of dry defatted tissue. All the determinations were made in two parallel experiments. The obtained results were analysed statistically according to Student's t test, taking differences for which $p<0.05$ as statistically essential.

## RESULTS

The results of the experiments which have been carried out are presented in table 1. In comparison with the control group in the medial and internal layer of the main aorta in patients who had died of vascular complications in the course of diabetes mellitus, no significant changes in the content of the total glycosoaminoglycans have been ascertained. However a considerable increase in the hialuronic acid fraction and a decrease in the chondroitine — 4 — sulphate content has been noted. Although no important discrepancies have not been observed in the remaining fractions, it should be pointed out that the group of sulphuric glycosoaminoglycans represented by heparan sulphate, chondroitine — sulphates and dermatane sulphate was about 70% of the total GAG in aortas of the control group and decreased down to about 67% in the

Table 1. Composition of glycosoaminoglycans of aorta's in intima-media layer control group and diabetic patients

| | Control group | | Diabetic patients | | Significant of differences | |
|---|---|---|---|---|---|---|
| | | | | | t | p |
| Number of patients | 10 | | 16 | | | |
| Age | 72.3 ±8.6 | | 67.4 ±8.4 | | | |
| Total GAG | 617.8 ±83.8* | 100** | 599.8 ±85.7* | 100** | 0,55 | NS |
| Non complexing fraction | 95.4 ±12.6 | 15.4 | 96.3 ±21.5 | 16.1 | 0,12 | NS |
| Hyaluronic acid | 46.8 ±8.7 | 7.6 | 60.6 ±9.1 | 10.1 | 2,38 | 0,05 |
| Heparan sulphate | 101.0 ±24.0 | 16.3 | 90.7 ±19.0 | 15.1 | 1,22 | NS |
| Chondroitin-4-sulphate | 139.1 ±12.9 | 22.5 | 120.7 ±14.3 | 20.1 | 2,83 | 0,01 |
| Chondroitin-6-sulphate | 104.9 ±24.2 | 17.0 | 114.4 ±15.3 | 19.1 | 1,24 | NS |
| Dermatan sulphate | 83.8 ±9.8 | 13.6 | 76.4 ±13.7 | 12.7 | 1,49 | NS |
| Heparin | 46.8 ±8.4 | 7.6 | 40.7 ±9.8 | 6.8 | 1,73 | 0,10 |

* in μg of uronic acids per 100 mg of dry defatted tissue (meau ±SD).
** %.

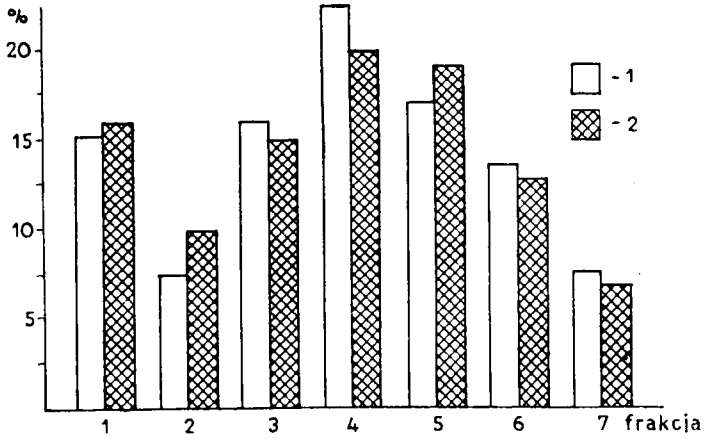


Fig. 1. Proportional composition of the fraction of glycosoaminoglycans of the intima-Media layer of aorta in investigated groups. Blank spaces — control group, printed spaces — patients who died in consequence of vascular complications in the course of diabetes

experimental group. In the intima-media layer of the experimental group a certain decrease in heparine content has been observed.

## DISCUSSION

Glycosoaminoglycans are a very active heterogenous group of polysaccharides which combined with proteins in the form of proteoglicans condition a number of properties and functions of the basic substance of the connective tissue (17). In the light of recent investigations there is no doubt as to their role in atherogenesis. It has been proved that glycosoaminoglycans form complexes with lipids, which in this are observed to the arterial wall, imitiating so colled metamorphosis in the smooth muscle cells, which is an early symptom of therosclerotic changes (2, 15).

The content and composition of glycosoaminoglycans in the vascular wall undergoes important changes in the process of physiological ageing in experimental sclerosis and in cases of a disturbance metabolism carbohydrate (7, 8, 12, 13, 18). In our previous publication denoting changes in the content and composition of glycosoaminogylcans in alloxan, diabetes and experimental sclerosis, an increase of glycosoaminoglycans has been observed (8). A similar increase in the hialuronic acid content after provoking alloxan diabetes was observed by I c h i d a (10). In the investigations carried out now, unlike in experiments on animals, no increase in the total glycosoaminoglycan content of the intima-media layer of the human aorta has been observed. However, as it can be seem from Fig. 1 presenting composition of glycosoaminoglycans in the control and experiment groups,

similarly as previously, in aortas of diabetic patients, the amount of hialuronic acid was increasing while the content of heparan sulphate chondroitine — 4 — sulphate and dermatane sulphate was decreasing at the same time.

The results of investigations by other authors denoting the composition of glycosoaminoglycans of aorta in the course of experimental *diabetes mellitus* also vary on that subject. According to Cohen and Foglia (4) experimental diabetes due to partial pancreatecomy initially caused an increase in the content of glycosoaminoglycans of aorta, including hialuronic acid and sulphuric glycosoaminoglycans, but in 6 months time their visible decrease was observed. In short-lasting experiments Brosnan *et al* did not ascertain an influence of a pancreatectomy on the glycosoaminoglycan content in aorta (3). It may be suggested that these differences may depend also on species specificity, the period of the ocurrence of disturbances in the carbohydrate metabolism and the age of investigated populations, which conditions the turnover of polysaccharides in the connective tissue (9).

The changes which we have observed denoting gylcosoaminoglycans of aorta wall in the course of long-lasting diabetes in humans may be partially explained by investigations on the influence of insulin on the metabolism of these compounds. As it can be seen from experiments by Urrutia *et al.* (20), Newmark *et al.* (14), and other authors (21), in contrast to other cells of the body insuline deficiency does not decrease the use of glucose by the cells of medical layer of aorta. Since, as it is well known, glucose is a precursor of both hexosamine and uronic acids which are the componentes of polysaccharide chains of glycosoaminoglycans (1), in diabetes the increased synthesis of these compounds may occur. Intensive incorporation of $^{14}C$ glucose to glycosoaminoglycans of aorta in rats suffering from alloxan diabetes (16) seems to be the confirmation of this fact. Since, on the other hand, there are some data indicating a stimulating participation of insulin in incorporation of sulphate to sub-unites of glycosoaminoglycans (5, 6),it can be assumed that its deficiency will results in the increase of hialuronic acid content and in the decrease of derivatives of sulphate. Such an interpretation does not exclude the influence of hormonal disturbancs on the polypeptide chain synthesis of proteoglycans (11).

The problem of the influence of changes in glycosoaminoglycans of aorta in the course of diabetes on the development of atherosclerosis is highly complicated. Apart from the disturbances in lipid metabolism (2, 15), taking into account the properties of hilauronic acid, it may result in the increased hydration of the arterial wall. This in turn may lead to the loosening of the layer of endothelial cells, may distrub the process

of selective absorbtion and decrease mechanical resistance of vascular wall. The above hypothesis requires further investigation.

Findings from the preformed experiments encourage us to draw the following conclusion:

In patients who died of vascular complications in the course of diabetes an increase in hialuronic acid content and a slight decrease in sulphuric glycosoaminoglycans in intimamedia layer of aorta can be stated.

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STRESZCZENIE

Przeprowadzono badania zawartości glikozoaminoglikanów całkowitych i ich frakcji w błonie środkowej i wewnętrznej aorty u osobników zmarłych z powodu powikłań miażdżycowych w przebiegu cukrzycy. W porównaniu z grupą kontrolną bez zmian miażdżycowych w grupie badanej stwierdzono statystycznie istotny wzrost zawartości kwasu hialuronowego oraz spadek zawartości chondroityno-4-siarczanu. Nie obserwowano istotnych zmian w całkowitej ilości glikozoaminoglikanów ani w pozostałych frakcjach tych związków.

РЕЗЮМЕ

Проведены исследования полных глюкозоаминогликанов и их фракций в средней и внутренней оболочках аорты у людей, умерших вследствие атеросклеротических осложнений при сахарном диабете. По сравнению с контрольной группой без атеросклеротических изменений, в обследуемой группе обнаружено статистически значительное повышение гиалуроновой кислоты и одновременное понижение содержания хондроитин-4-серной кислоты. Не наблюдалось значительных изменений ни в общем количестве глюкозоаминогликана, ни в остальных фракциях этих соединений.