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A METHOD OF QUANTITATIVE DETERMINATION OF GLYCOSAMINOGLYCANES (MUCOPOLYSACCHARIDES) IN ANIMAL TISSUES AND SOME ORGANOPREPARATIONS

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SUMMARY

A unified, highly sensitive method is suggested which can be applied to determine the level of glycosaminoglycanes in raw materials (vitreous body, duodenum mucous membrane, eye cornea, hyaline cartilages, lungs) at individual processing stages, as well as to control the quality of the finished product containing glycosaminoglycane as a biologically active component.

INTRODUCTION

At present many researchers are taking an interest in the problem of evaluating the quantitative level of glycosaminoglycanes (GAG) in animal organs and tissues, their extracts and in medicines in which GAG are pharmacologically active components.

A physico-chemical methods of GAG determination requires highly purified standard substances. There is a Standard of WHO on heparin activity determination but it does not cover heparin content measurement. From literature it is known that, to quantitatively determine chondroitin sulfate, many scientists use the SIGMA chondroitin sulfate (99%) as a standard.

It is common to measure the GAG content by the level of hexosamines and uronic acids. The uronic acids/hexosamine ratio allows to identify GAG (heparin, chondroitin sulfate, hyaluronic acid).

Due to the nature of monosaccharide components and to covalent links between carbohydrates and proteins, the chemical approach to GAG differs in many respects from the methods used to study heteropolysaccharides or simple proteins.

METHODS

At present, to release sugars from GAG, acid hydrolysis is nearly universally applied as glycoside links are usually stable to alkalies. Acetyl glucosamine release follows two courses (Fig.1) (1). Hydrolysis II is both slow and results in an incomplete release of acetyl glucosamine. Therefore for each GAG such conditions are chosen which contribute to the maximum yield of acetylglucosamine and to the minimum splitting of the amide link (hydrolysis I). This scheme is discussed in detail by Johnson (2); it was shown that the above requirements are met by using 4 N HCl for 3 hr at 100°C. Depending on the GAG kind, only temperature can be changed. The method of hexosamine determination is based on the widely used Morgan-Elson reaction in which the amino sugar resulting from GAG hydrolysis with HCl reacts with an alkali and the products formed are condensed with the Erlich reagent to form a purple-coloured mixture of pyrrols (the optical density maximum at 530 nm) (3,4,5).

Uronic acids in GAG are determined according to Dische (6,7) and the modifications of his method (8,9,10). The method involves the interaction of the carboxylic groups of uronic acids, resulting from hydrolysis in sulphuric acid, with carbazole. The reaction results in specific violet-pink colouring with the absorption maximum at 530 nm. It was noticed that at low concentrations of uronic acids the maximum colour development is achieved quicker and the colour is more stable. The error of the method is ±0.82%.

The present experiments were carried out to develop quantitative methods for determining GAG in medicines, v iz., in heparin, chondroitin sulfate and in vitreous body of the domestic production, in keratin-sulfate from beef and pork eyes; as well as GAG isolated from pork duodenum. For this, optimum conditions for hydrolysis were chosen which ensure the maximum yield of acetylglucosamine and the maximum splitting of the amide link.

Such optimum conditions were as follows:
- for heparin and duodenum GAG: a 10 mg test portion, hydrolysis with 4 N HCl for 3 hr at 115°C;
- for chondroitin sulfate: similar conditions but 120°C;
- for keratin-sulfate from animal eye cornea: a 100 mg test portion, 5 ml of 4 N HCl, hydrolysis time 18 hr, temperature - 120°C;
- for vitreous body (hyaluronic acid): 4 ml of the preparation, hydrolysis with 8 N HCl (4ml) for 3 hr at 118°C.

A study into the relation of the maximum yield of uronic acids to the conditions of sample preparation for the analysis indicated a possibility of determinations in heparin and chondroitin sulfate without pre-hydrolysis. Thus, hydrolysis of 10 mg of heparin with 5 ml of 4 N HCl for 3 hr at 105°C-110°C yields 18-20% of uronic acids, without pre-hydrolysis the yield is 33-35%. A similar regularity is also characteristic of chondroitin sulfate.

The results of the determination of hexosamine and uronic acids levels are given in Fig. 2.

The results obtained showed that their levels in heparin are 18 and 34%, respectively. The ratio of uronic acids to hexosamine is 2:1. Hexosamine in chondroitin sulfate was 27% and the uronic acids to hexosamine ratio was 0.97. Hexosamine content in the vitreous body was 21% for cattle, 10% for pigs, 12% for sheep and goats.

RESULTS

The methods for measuring hexosamine and uronic acids described above allow to objectively evaluate the quality of raw materials and of medicinal preparations, to establish the influence of individual processing stages upon the yields of these biologically active components.

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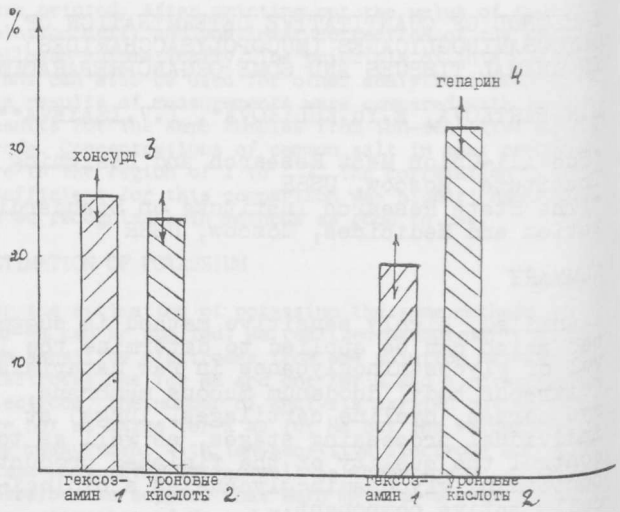

Fig.1. The level of hexosamine and uronic acids in chonsurd and heparin: 1 - hexosamine, 2 - uronic asids, 3 - chonsurd, 4 - heparin.

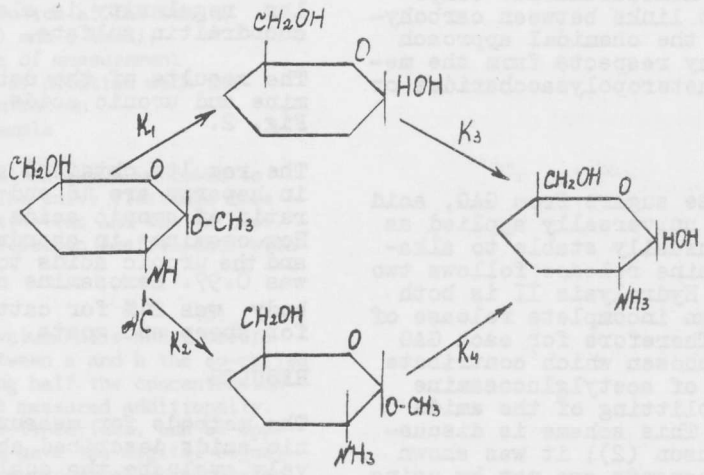

Fig.2. A scheme of acetylglucosamine release during hydrolysis.