THE EFFECT OF CHONDROITIN SULFATE (CHONSURIDUM) ON COLLAGEN IN VIVO

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We here report a study of the effect of acid mucopolysaccharide chondroitin sulfate on regeneration in vivo.

The theoretical basis for the clinical application of chondroitin sulfate in cases where wounds have remained unhealed for a long time was the phenomenon previously reported of disturbed relationships between components of the enzyme-substrate systems hyaluronidase-hyaluronic acid and chodroitin sulfuric acid [1]. In various pathological conditions, the reaction may be shifted either so that there is an accumulation of acid mucopolysaccharides (in cicatrization, hematoma, tissues of a false articulation, etc.) or the change may be such as to reduce the amount of these components. It has been found that in wounds which remain unhealed for a long time and which contain soft granulation tissue, the amount of mucopolysaccharides is reduced. This circumstance was the reason for developing the standard preparation of chondroitin sulfate for stimulating the repair processes in wounds which heal with difficulty.

The preparation chonsuridum† was obtained by our modification of Einbinder and Shubert's method [5], which enabled a sufficiently high degree of polymerization of the molecules and minimal loss of protein into the solution during extraction to be attained. The preparation was identified by various methods: 1) by electrolytic separation using the method of Rienits [10]; 2) by a turbidity method, using the method of Mathews and his co-workers [7]; 3) by determining the hexosamine content, by the method of Elson and Morgan as modified by Boas [4]; 4) by separating with ion exchange resins using Gardell's method [6].

We here give the hexosamine and nitrogen content of chondroitin sulfate and, for comparison, the results of several others authors who separated it under laboratory conditions using various more complex methods (see table).

When the preparation was separated using the ion exchange resin Dowex-50, it was found that of the total hexoseamines 85% was galactosamine, and 15% was glucosamine (Fig. 1).

In clinical practice, favorable results were obtained by using chondroitin sulfate in cases of operative wounds showing granulation tissue, and trophic abscesses; less good results were obtained after compound fractures, particularly when they were complicated by osteomyelitis, and in third degree burns. No adverse side effects were noted.

The general reaction of the organism to the local external application of the preparation was controlled by determining the protein fraction of the blood serum by paper electrophoresis, the glucoprotein fraction from serum mucoid using Winzler's method [11] as modified by Menini and his co-workers [8], and the sial acids using the

* Deceased.

† The preparation was made by B. S. Kasavina and G. D. Zenkevich of the Central Institute of Traumatology and Orthopedics in collaboration with M. Z. Alfer'eva and L. S. Pozhariiskaya from the Scientific Research Institute of the Meat Industry and by A. O. Pashkov, S. S. Togunov, and T. S. Bogushevich of the Leningrad Pharmaceutical Works.

Analysis of a Preparation of Chondroitin Sulfate

Source	Hexoseamine	Nitrogen	Author
	%		
Cartilage (tracheal and nasal)	23.7-27.0	2.3-3.2	Kasavina, Zenkevich, et al. [1961]
Cartilage	24.1-31.1	2.4-3.2	Meier and co-workers [1956]
"	26.3	–	Muir [1956]
Cartilage (tracheal)	28.07	2.13	Rodgers [1951]
"	23.55	2.15	Einbinder and Shubert [1950]
Cartilage	28.7	3.3	Braint [1958]
"	22.4-26.0	5.0-6.4	Matthews and Louzeitl [1958]
Suckling rat skeleton	20.4	2.86	Dzivatkovskii [1956]

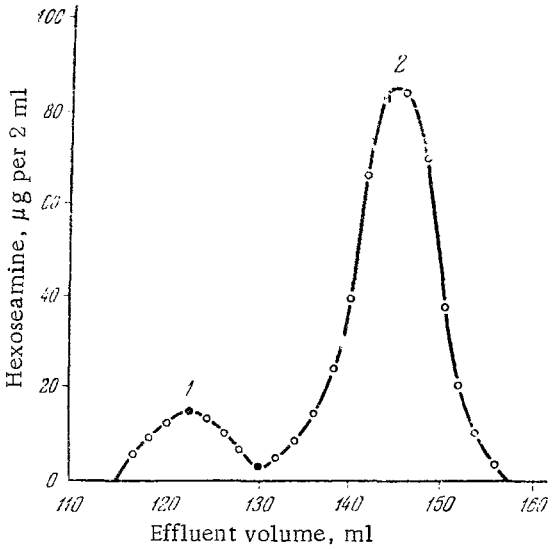

Fig. 1. Division of chonsuridum on an ion exchange membrane Dowex-50 into glucosamine (1) and galactoseamine (2).

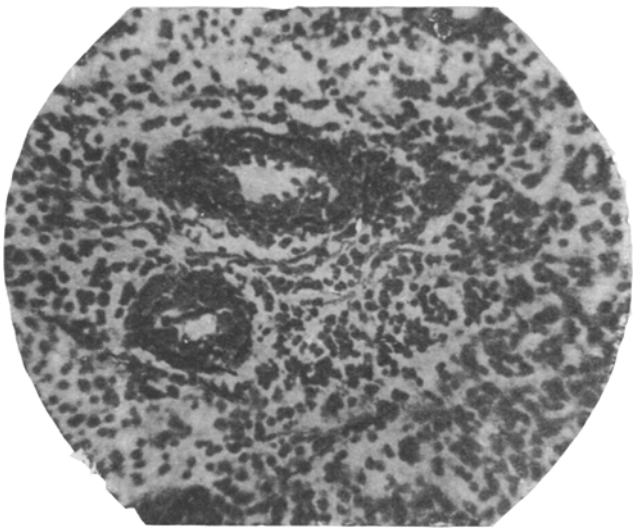

Fig. 2. Collagenous fibers around the vessels after the third day of treatment with chonsuridum (patient B); stained with azan; magnification 500 ×.

method of Niazia and Steit as modified by Larskii [3]. These indices, which indicate an inflammatory reaction very sensitively, remained within normal limits during recovery.

An attempt was made in clinical cases to study the means whereby the preparation influences the healing of wounds, and histological, histochemical, and cytochemical methods were applied. Before the preparation was used, only occasional precollagenous and collagenous fibers could be observed distributed around the capillaries in the collagenous tissue (Fig. 2). There was only a very small amount of ribonucleic acid in the endothelial cells; in other cells of the connective tissue there was no ribonucleic acid.

After 1-3 dressings with chondroitin sulfatehad been applied (on the 3rd to 10th days), the granulations contained a considerable number of capillaries which were directed toward the surface of the wound. There was an increase of connective structures between the vessels. The amorphous intercellular substance was markedly metachromatic. In it, fine precollagenous and collagenous fibers appeared; a large number of these fibers were also seen around the capillaries (Fig. 3).

The cells of the connective tissue showed a strong ribonucleic acid reaction.

Before healing, the granulation tissue was noticeably compact (the walls of the vessels were thickened), the amount of intercellular amorphous substance was reduced, it was less metachromatic, and there was a further increase in the amount of precollagenous and collagenous fibers. The connective tissue cells (fibroblasts, fibrocytes, and histiocytes) showed a less active ribonucleic reaction.

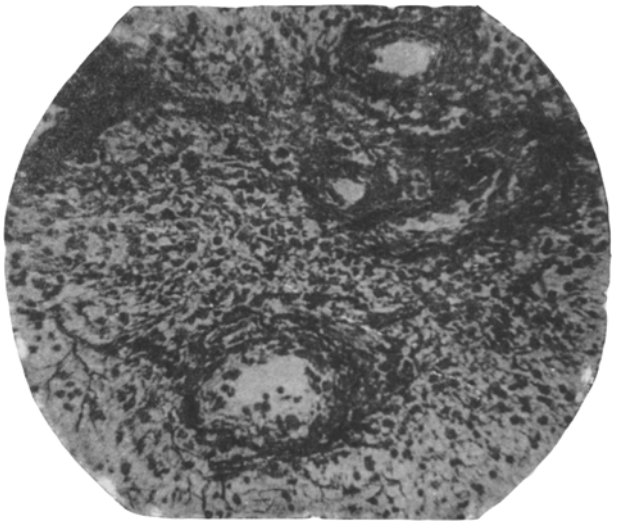

Fig. 3. Massive formation of collagenous fibers around the vessels and in the ground substance of the connective tissues after ten days' treatment with chonsuridum (patient B); stained with azan; magnification 500 ×.

A control wound treated with testicular and streptococcal hyaluronidase showed clearly that both hyaluronic and chondroitin sulfuric acid are present in the granulation tissue.

The results of these investigations show that local clinical application of chondroitinsulfate stimulates the regeneration of connective tissue cells and aids the synthesis of the proteins of precollagen and collagen. Ribonucleic acid plays an important part in this process.

SUMMARY

A study was made of the effect of acid mucopolysaccharide chondroitinsulfate on the healing of wounds. A standard preparation (chonsuridum) was tested for electrophoretic mobility, for turbidity and hexoseamine content and by chromatographic separation on ion exchange resins. The preparation was found to stimulate regeneration in slowly healing wounds. The action of chondroitinsulfate was studied histochemically. Local (external) application stimulated cellular connective tissue regeneration and the synthesis of precollagen and collagen proteins. Ribonucleic acid plays an important part in this process. The best clinical results were obtained in treating slowly granulating wounds after surgical intervention, decubitus ulcers, trophic ulcers etc.

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