HYALURONIC ACID IN HEBERDEN'S NODES

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(RECEIVED FOR PUBLICATION MARCH 6, 1957)

Introduction

Since Heberden (1803) wrote about *digitorum nodi*, these nodes on the terminal interphalangeal joints have been considered to comprise a distinct form of joint disease, which in later years has been classified as a form of osteo-arthritis. Stecher (1955), in an excellent summary of his extensive researches, distinguished the occasional post-traumatic node from the more common idiopathic nodes which he concludes are genetically determined. Although Stecher is of the opinion that Heberden's nodes are not associated with similar joint changes in other parts of the body, this view is not universally accepted (Kellgren and Moore, 1952) and recent radiological surveys of samples of the general population have shown a highly significant association between Heberden's nodes and radiological signs of osteo-arthritis in other joints (Kellgren, 1956). This communication is solely concerned with the pathogenesis of Heberden's nodes and presents no new evidence on the controversy about Heberden's nodes and generalized osteo-arthritis.

Heberden's nodes usually develop slowly and painlessly over many years, but in some patients the process had a rapid evolution so that massive bony outgrowths may develop in a single year. The formation of such acute nodes is accompanied by much spontaneous pain and the development of small cystic swellings over the dorsum of the joint. These swellings occasionally burst to discharge clear gelatinous material.

It seems probable that this gelatinous material is in some way connected with the pathogenesis of the nodes and the mechanism of pain production in such cases. In a previous study (Addis, Jepson, and Kellgren, 1950) and in later observations, we have noted that the pain in acute Heberden's nodes increases rapidly in severity during short periods of arterial occlusion and that this increase is immedi-

ately relieved when the circulation is restored. After the evacuation of the contents of such a cyst, the node gradually becomes painless. The chemical nature of the gelatinous material in these cysts is of some theoretical interest, and we have therefore studied material obtained from four typical cases of acute Heberden's nodes.

Clinical Cases

All four patients were women aged 53 to 61 years. Their symptoms were of 2 to 5 years' duration, and all of them complained of painful swellings of the terminal interphalangeal joints with cyst formation and occasional discharge of clear gelatinous material. In all cases the painful phase was followed by massive bony enlargement of the affected joint. This process affected first one finger and then another, so that at the time of examination all the patients had many fully developed bony nodes as well as one or two painful cystic swellings. In all the patients there was some involvement of the proximal interphalangeal and the first carpo-metacarpal joints, and in three of them there were signs of osteo-arthritis in other joints, such as the knees, shoulders, spine, or great toes, but in all of them the hands were the parts predominantly affected. None of the patients had psoriasis or pitted nails.

Radiographs of the hands showed typical osteo-arthritic changes, but there was no osteoporosis or articular erosion of the rheumatoid type.

In each case 0.2 to 0.5 ml. of clear gelatinous material was obtained from a painful cystic swelling of the dorsum of a distal or proximal interphalangeal joint. The material was too viscous to aspirate and it has to be evacuated by making a small incision under anaesthesia provided locally by an ethyl chloride spray or by a procaine ring block at the base of the finger. A bloodless field was provided by a finger tourniquet.

Methods of Analysis

With such small amounts of material, quantitative analysis was not possible. The fluids were analysed qualitatively for free and bound proteins and for

mucopolysaccharide. The material which gelled at room temperature was diluted with saline and aliquots used for the various analyses.

Protein Analysis

(a) Electrophoresis was carried out on filter paper (Whatman No. 1) in a horizontal type apparatus in veronal buffer pH 8.6, $\mu=0.05$, and the proteins were stained by the method of Durrum (1950). Diluted human serum was run simultaneously; volumes of 50-100 μ l. were applied to the paper.

(b) Various qualitative protein reactions were applied including Millon's, ninhydrin, and xanthoproteic reactions.

(c) Samples were hydrolysed at 100° C. in 6N-HCl for 24 hrs, and the hydrolysate was examined by two-dimensional chromatography for amino acids using butanol-acetic acid-water (1 : 1 : 1) and phenol-water solvent systems.

Mucopolysaccharide Analysis

(a) Electrophoresis was carried out in acetate buffer pH 5, $\mu=0.05$, and the papers were stained with Alcian blue and toluidine blue. Authentic samples of hyaluronic acid from human umbilical cord and chondroitin sulphate from bovine tracheal cartilage were run simultaneously.

(b) Samples were incubated with both testicular hyaluronidase (Benger's "Hyalase") and streptococcal hyaluronidase (kindly provided by Dr. J. H. Humphrey), and the reaction mixture was tested for reducing substances by the method of Somogyi (1945).

(c) The mucopolysaccharide in the fluids from Cases 3 and 4 was isolated, using cetylpyridinium chloride (Scott, 1956). The diluted fluid was dialysed against 0.2 M Na₂SO₄ for 48 hrs, and 1 per cent. cetylpyridinium was added. No precipitate was obtained after standing at 30° C. for several hours, indicating the absence of chondroitin sulphate. The solution was diluted 1 in 5 with water, and stood again at 30° C. for several hours. The precipitate obtained was washed with dilute acetic acid and analysed for protein and mucopolysaccharide.

Results

Protein Analysis.—In Samples 1 and 3, no protein was detected, nor were any amino acids detected after hydrolysis. Small amounts of protein were present in Samples 2 and 4, but these were contaminated with blood which was the probable source of protein. Electrophoretic analysis showed only traces of albumin and γ globulin.

Mucopolysaccharide Analysis.—Only one band was obtained on electrophoresis. This moved

slowly and corresponded to that obtained with an authentic sample of hyaluronic acid. The bands stained with Alcian blue and orthochromatically with toluidine blue. Reducing sugars were released by both testicular and streptococcal hyaluronidase, indicating the presence of hyaluronic acid (Meyer, 1947).

The mucopolysaccharide isolated from Samples 3 and 4 were also hydrolysed by both enzymes and no amino acids were released on hydrolysis. No sulphate could be detected in the hydrolysate. Hence this material appeared to be hyaluronic acid free of protein or peptide.

Discussion

The results suggest that the fluid from Heberden's nodes contains only hyaluronic acid free of protein or peptide. This is in contrast to the findings in synovial fluid from a patient with generalized osteoarthritis, which contained considerable amounts of serum type proteins (unpublished data). The hyaluronic acid precipitated with cetylpyridinium chloride from the fluid from Case 3, which was contaminated with blood, was also protein-free, whereas cetylpyridinium chloride precipitates a hyaluronic acid protein complex from bovine synovial fluid (Ogston and Blumberg, 1957).

The significance of this local accumulation of hyaluronic acid is not yet clear, but it is presumably related in some way to the peculiar painful state found in acute Heberden's nodes, and it may also play a part in the bony hyperplasia which is such a feature of this form of osteo-arthritis.

Summary

The gelatinous fluid from the Heberden's nodes from four cases of generalized osteo-arthritis has been analysed qualitatively.

The fluids were examined for mucopolysaccharides and proteins by electrophoresis and by chromatography after hydrolysis in strong acid. Samples were incubated with testicular and bacterial hyaluronidase, and in two cases the mucopolysaccharide was isolated using cetylpyridinium chloride and subjected to analysis.

The results indicate that the Heberden's nodes contain only hyaluronic acid free of either bound or free protein.

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L'acide hyaluronique dans les nodosités d'Heberden**RÉSUMÉ**

Le liquide gélatineux de nodosités d'Heberden, provenant de quatre cas d'ostéoarthrite généralisée, a été analysé qualitativement.

La recherche des mucopolysaccharides et des protéines dans ces liquides fut effectuée par électrophorèse et par chromatographie, après hydrolyse dans un acide fort. Des échantillons furent incubés avec de l'hyaluronidase testiculaire et bactérienne et, dans deux cas, le mucopolysaccharide fut isolé à l'aide de chlorure de cétilypyridinium et soumis à l'analyse.

Les résultats montrent que les nodosités d'Heberden contiennent de l'acide hyaluronique, sans protéine, libre ou liée.

El ácido hialurónico en las nudosidades de Heberden**SUMARIO**

El líquido gelatinoso de las nudosidades de Heberden, procedente de cuatro casos de osteoartritis generalizada, fué analizado cualitativamente.

Se investigaron los mucopolisacaridos y las proteínas en estos líquidos por medio de electroforesis y cromatografía, después de hidrolisis en ácido fuerte. Los especímenes fueron incubados con hyaluronidase testicular o bacteriana y, en dos casos, el mucopolisacarido fué aislado con la ayuda de cloruro de cetilpiridinium y sometido al análisis.

Los resultados indican que las nudosidades de Heberden contienen ácido hialurónico, sin proteína, libre o ligada.