CHANGES IN PERMEABILITY OF ARTICULAR CARTILAGE IN EXPERIMENTAL LATHYRISM

BY

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Articular cartilage does not contain blood vessels, the nutrition of chondrocytes being derived mainly by diffusion of synovial fluid (Stockwell and Barnett, 1964; Ekholm, 1951). It has been demonstrated that the permeability of articular cartilage diminishes with ageing (Stockwell and Barnett, 1964; Tsaltas and Kyriazis, 1967). This change has been attributed not only to the physical changes brought about by the passage of time, but also to modifications of the chemical constituents of the matrix (Kaplan and Meyer, 1959). Verzar (1963), using mechanical and chemical means, suggested that one of the principal changes associated with ageing is the increase in cross-linking of collagen fibres. He interprets this to mean that there is an increase in the degree of maturation of this protein. Experimental lathyrisms, on the other hand, is characterized by a lack in the degree of maturation of collagen. This has been demonstrated by an increase in lathyrotic tissues of immature neutral salt-soluble collagen (Levene and Gross, 1959; Gross and Levene, 1959). On the basis of these observations, it has been suggested that experimental lathyrisms is the apparent antithesis of ageing in connective tissue (Bickley, 1964). The purpose of this study was to test this hypothesis, that lathyrisms is indeed the antithesis of ageing, and to determine what implications a change in cartilage permeability in experimental lathyrisms might have on the pathogenesis of this disease.

Material and Methods

51 male Swiss mice weighing 12-15 g. were used, eighteen as controls and 33 as experimental animals. The treated animals received a daily subcutaneous injection of 5-30 mg. buffered aminoacetonitrile sulphate

(AAN), pH 7.4. The controls received an equivalent amount of buffered sodium sulphate.

All animals were killed on the seventh day by manual separation of the cervical vertebrae. The proximal head of the femur was immediately removed and immersed for 5 min. in a silver proteinate, 5 per cent. Protargol-S (Winthrop Laboratories, New York, N.Y.) according to the method of Stockwell and Barnett (1964). The tissue was then fixed in neutral buffered formalin, decalcified in 5 per cent. formic acid and sodium citrate, embedded in paraffin, and sectioned at 6 microns. The penetrated silver was reduced and developed by the method of Bodian (1936). Aniline blue was used as a counterstain. The depth of penetration was measured with an ocular micrometer at a magnification of 450 $\times$. A second group of sections was stained with Alcian blue, pH 1.0, and a third with the colloidal iron technique.

Results

Treatment with AAN produced a significant decrease in the permeability of articular cartilage by the silver salts. It was found that all the dosages of AAN used produced symptoms of the disease, but did not increase or decrease the range of silver penetration in the treated group. The Table shows the mean penetrations and the application of the "t" test. The mean penetration in the AAN-treated mice was 55 microns compared to 75 microns in the untreated animals. Fig. 1 shows the appearance of Protargol penetration in a typical preparation.

TABLE
AVERAGE PENETRATION OF PROTARGOL INTO
ARTICULAR CARTILAGE IN MICE TREATED
WITH AAN AND IN CONTROLS

Series	No. of Mice	Mean Penetration (μ)	Standard Deviation
Controls	18	75	± 10.4
Experimental	33	55	± 11.7

"t" = 7.66, the penetration being significantly greater in the control animals.

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The histochemical demonstrations of sulphated acid mucopolysaccharides by Alcian blue and colloidal iron were quite similar. Fig. 2 shows sections stained with Alcian blue. Note that the staining is rather uniform in the control animals, while the treated animals appear to have a concentration of Alcian blue-positive material close to the articular surface.

Discussion

On the basis of permeability this study does not support the concept that administration of lathyrictic agents to animals affects collagen cross-linking in a

manner which may be interpreted as the apparent antithesis to ageing, since the collagen maturational defect found in experimental lathyrism did not increase cartilage permeability. The decrease in permeability found in this study parallels that found in papain-treated animals where the apparent degradation of the protein moiety of protein-polysaccharide complex takes place (Thomas, 1956). This finding of decreased permeability suggests a defect in lathyrism of complex formation between non-collagenous protein and mucopolysaccharides as reported by Kennedy and Kennedy (1962). Since chondroitin sulphate-protein macromolecules exhibit a surface attachment to collagen fibres (Serafine-Fracassini and Smith, 1966), and this

Fig. 1.—Protargol penetration, proximal femoral articular cartilage. Control left, lathyrictic specimen right. $\times 100$.

Fig. 2.—Distribution of sulphated acid mucopolysaccharides in mouse articular cartilage. Control left, lathyrictic specimen right. Alcian blue. $\times 40$.

binding is presumed to be electrostatic (Einbinder and Schubert, 1951), the collagen defect in experimental lathyrisms may indirectly affect the mucopolysaccharide non-collagenous protein complex. The accumulation of Alcian blue-positive and colloidal iron-positive material at the periphery of the articular surface in the experimental animals suggests an unmasking of reactive anionic sites in this area, or a breakdown and migration of protein polysaccharides to this locus in the process of diffusion through the articular surface. This accumulation of Alcian blue and colloidal iron-positive material at the periphery of the articular surface supports the contention that the ultimate loss of chondroitin sulphate from the matrix may be due to depolymerization. The reflection of the loss of chondroitin sulphate is seen in the increased mucoprotein and hexosamine levels in the blood of lathyrotic animals (Schwartz, 1959). The depolymerization may also explain the increase in metachromasia and Alcian blue staining in lathyrotic cartilage reported by Karnovsky and Karnovsky (1961), probably reflecting an increase in the number of anionic groups available for binding. The dense, fixed negative charge in the articular surface might tend to repel the anionic silver-proteinate in much the same way as a charged membrane, and might thus limit its penetration. In the normal situation the anionic groups of mucopolysaccharides are either free or associated with simple inorganic cations, giving cartilage the

properties of a cation exchange resin (Boyd and Newman, 1951). The uniform distribution of these sites in the normal situation may allow the silver proteinate some degree of penetration. Another possibility was suggested by Matukas, Panner, and Orbison (1967), who showed an increase in the diameter of lathyrotic collagen and an enhancement in its ability to bind cationic and anionic stains. Collagen in this state may exert an additional effect, not only because of the increase in apparent ionic sites, but by acting mechanically to prevent the penetration of the silver-proteinate.

The absence of blood vessels in articular cartilage and the speculation that nutrition in this area is supplied by simple diffusion suggests that the profound effects on the metabolism of cartilage cells by lathyrigenic agents may be due in part to the inability of charged molecules, principally anionic metabolites, to reach the area or return from it.

Summary

Results of this study show a marked decrease in permeability of lathyrotic mouse femoral articular cartilage compared with controls when measured with silver proteinate. The results do not support the concept that administration of lathyrotic agents to animals affects collagen cross-linking in a manner which may be interpreted as the apparent antithesis to ageing.

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Les changements dans la perméabilité du cartilage articulaire dans le lathyrisme expérimental

RÉSUMÉ

Les résultats de cette étude montrent une diminution marquée de la perméabilité du cartilage articulaire fémoral de la souris exposée au lathyrisme expérimental par comparaison aux témoins quand le protéinate d'argent est employé. Les résultats ne soutiennent pas le concept que l'administration des agents de lathyrisme aux animaux affecte le "cross linking" du collagène d'une façon qui pourrait être interprétée comme une antithèse apparente au vieillissement.

Cambios en la permeabilidad del cartilago articular en el latirismo experimental

SUMARIO

Los resultados de este estudio muestran una marcada reducción en la permeabilidad del cartilago articular femoral del ratón latirítico, comparado con testigos, cuando son medidos con proteinato de plata. Los resultados no apoyan el concepto de que el administrar agentes latiríticos a animales afecta el intereslabonamiento colagenoso de un modo que pudiera interpretarse como aparente antítesis del envejecimiento.