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BIOLOGICAL ASPECTS OF THE PHYSIOLOGY OF HYALINE CARTILAGE

BY

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Our present incomplete understanding of the biology and physiology of cartilage is built almost entirely on an earlier morphological basis and does not pay sufficient attention to the polysaccharide present in the intercellular medium of the cartilage. The unique structure and physico-chemical properties of chondroitin sulphuric acid justify a critical examination of our theories on the subject.

Native chondroitin sulphuric acid is a polymerisation product, a long chain molecule with high molecular weight. It is probably combined with a protein in the cartilage. The aqueous solution is viscous and shows marked susceptibility to alkalis and other substances. Even small variations in the pH cause a breakdown of the molecule. The native substance has been obtained in small amounts by careful extraction in salt solution by Mayer, Blix and Snellman and others. When the native compound is broken down, chondroitin sulphuric acid is obtained and consists of a carbohydrate complex, with a strongly acid reaction. The sulphate in ester linkage makes it possible to demonstrate the molecule histochemically by the metachromatic staining reaction. One can even, under certain conditions, obtain a rough quantitative estimation of the amount of chondroitin sulphuric acid present in sections of cartilage.

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This unique molecule is a characteristic constituent of the intercelluar substance of cartilage, and I shall try to give an account of our present knowledge of its biology.

It is usually believed that it is synthesised by the chondrocytes, but proof is lacking. Hansen did not venture any definite opinion; since then Clark and Clark, among others, have suggested that it might be formed by some process in the intercellular substance. Nothing definite is known about its genesis. This question is intimately connected with the metabolism of ester and other sulphates in cartilage.

During early foetal development, when a pre-cartilage begins to differentiate from the mesenchyme, a sulphuric ester acid accumulates, and this is the first detectable evidence that cartilage is beginning to develop. The foetal cartilage quickly develops a protecting connective tissue membrane and within this closed structure the further growth and differentiation of cartilage takes place. In the beginning the foetal cartilage is poor in intercellular substance, but this increases with growth until the proportion of cellular and intercellular substances characteristic for the different cartilage types has been reached. Little is known about the actual amounts of chondroitin sulphuric acid in the intercellular substance during foetal development. To judge from staining reactions it seems to be not unlikely that embryonic cartilage has nearly as high a local concentration of the acid in the intercellular substance as adult cartilage. Available chemical analyses have shown that each cartilage type contains a certain normal amount of chondroitin sulphuric acid; the greatest amount has been found in adults. These percentage figures have, however, only a limited value, since the figures do not express the local and regional variations within the different parts of the intercellular substance. This kind of information would be of considerable value and could be obtained by histochemical methods. As an example can be quoted the fact that there is usually less of the acid in the superficial zone of the joint cartilage than in the underlying central parts, and that its maximum concentration seems to

be near the surface of the chondrocytes. Both these facts are unexplained.

The decrease in chondroitin sulphuric acid with age must be placed in relation to a number of other changes which afflict cartilage. Thus, for example, there is a steady decrease in the number of cells from childhood throughout life, while at the same time there is an increase in the amount of calcium salts and the appearance of progressive degenerative processes, accompanied by calcification, fibrous metaplasia and the deposition of pigment. Reduced mechanical function may also be accompanied by changes in both the metabolism and the degree of differentiation. The observation of loss of chondroitin sulphuric acid has been explained by reduced synthesis by the chondrocytes, parallel with age-changes, but the real explanation is expected to be more complicated.

The intercellular substance of cartilage forms a gel containing normally about 70 % water. The water content is a function of the chemical composition and physico-chemical properties of the constituents of the gel. Changes in these and in the relative proportions of chondroitin sulphuric acid, proteins and other electrolytes also bring about changes in the water content. The varying water content can be considered to be a secondary phenomenon, an indication of the varying composition and condition of the gel. An isolated study of the water content of cartilage alone is of less interest and very difficult to explain in all details.

Chondrocytes are thought to obtain their nutrition by diffusion of nutrient substances from the surrounding tissues via the intercellular substance. The cartilage of a joint can be supplied either from the underlying bone, or via the synovia, or by both routes. However, nothing definite is known about the assumed diffusion routes, or about the diffusion mechanisms. Moreover, the physico-chemical factors governing diffusion—of both macro- and micromolecular substances—are unknown. In other words, we know that nutrition is satisfactory in healthy cartilage, but we do not know, how it is achieved.

The nutrition routes mentioned appear to be vulnerable, but little is known about morphological changes due to impaired nutrition. The maintenance of a joint's function seems to be a factor of the greatest importance, and Bloom suggested as early as 1938 that increased or diminished function of the joint would be sufficient to make the chondroitin sulphuric acid disappear from the joint cartilage.

Knowledge of the metabolism of cartilage is minimal. Studies of the glycolysis and oxygen consumption of joint cartilage have shown that a progressive cell degeneration occurs with increasing age. One is also forced to accept that metabolism of chondroitin sulphuric acid occurs in normal cartilage; a breakdown and subsequent resynthesis, but nothing is known. Incomplete studies on the effect of malnutrition, thyroidectomy, and on the role of pituitary hormones have not led to any definite conclusions. The pathologically hypertrophic epiphyseal cartilage which occurs in rickets has been shown to have the same content of chondroitin sulphuric acid and the same pH as normal epiphyseal cartilage. In scurvy new formation of the acid should be impaired, but this subject has not been sufficiently elucidated.

The fundamental problems of the origin and replacement of the acid form a suitable subject for further study with the help of isotopes technique. It is tempting to believe that the closely related hyaluronic acid and its enzyme-system hyaluronidase could play a part. If the enzyme could be demonstrated also in the synovia the low content of chondroitin sulphuric acid in the superficial layer of the joint cartilage would be explained.

If small fragments of cartilage are cultured in vitro, dedifferentiation to a fibroblast-like cell- type occurs, and there is a rapid loss of chondroitin sulphuric acid. But if a whole piece of embryo cartilage with perichondrium is cultured, a slow growth of normal cartilage takes place with preservation of the acid. In this way the growth of embryonic cartilage, its differentiation and ossification etc., have been studied. If cartilage fragments are cultured with fibroblasts the cartilage de-

generates and is digested by the fibroblasts. This observation, which was made by Roulet, is of interest for the functional pathology. It is significant that the usual culture media could not satisfy the nutritional requirements of cartilage. They have generally a weak alkaline reaction, and in view of the great susceptibility of chondroitin sulphuric acid to alkali one can readily understand that the environmental conditions in vitro are unsuitable. Nature herself carries out the tissue culture of cartilage more successfully as intra-articular foreign bodies often surrounded by a thick layer of normal hyaline cartilage. The nutritive conditions in the synovia are evidently quite sufficient for both growth and preservation of normal cartilage for years.

The normal ossification of hyaline cartilage is initiated by a disappearance of the chondroitin sulphuric acid and the disappearance already begins in the "hypertrophic" vesicular cartilage. In all places where there is considerable osteoblastic activity the polysaccharide has already disappeared. It is probably broken down, either by the shift in pH or by the action of enzymes from the primitive bone marrow. The removal of the acid can be regarded as a prerequisite for the establishment of an alkaline medium, which, in its turn, is necessary for the activation of alkaline phosphatase.

Recent investigations into the early changes occurring in cartilage during various acute and chronic inflammatory conditions show that also here a disturbance of the chondroitin sulphuric acid is the earliest detectable change. The breakdown begins round the inflammatory foci and is probably a result of the alkaline reaction. Menkin's studies have shown that inflammation requires an alkaline medium, and thus the acid seems bound to disappear. This observation also partly explains the great resistance of joint cartilage to infections. The possible interpretation of the alkali-binding function of the acid, and the part played by hyaluronidase produced by bacteria, etc. will not be discussed here. The resistance to infection was earlier attributed to the cartilage's lack of vessels, but this explanation does not seem adequate. In brief, an inflam-

matory focus implies the presence of a new and different medium for the cartilage, and other chemical factors which can alter the cartilage's physiology. Similarly, the chondroitin sulphuric acid disappears very early when cartilage is infiltrated by cancer and its supporting connective tissue.

Data obtained so far show significantly that healthy hyaline cartilage has a high concentration of chondroitin sulphuric acid in its intercellular substance. We have therefore reached the stage where it is necessary to analyse the properties of the acid in order to discover its functional significance, and to draw comparisons with other highly specialised tissues, such as the cornea and the umbilical cord.

1. *The molecular structure.* A long-chain molecule could be thought to be important in diffusion and nutrition. Barcroft and his co-workers found that the mucoid tissue of the umbilical cord acted as an extravascular transport route between the placenta and the foetus. It is conceivable that the mucoid of the cartilage could have a similar function in the transport of nutritient substances and water. Further, according to Hirsch, the chondroitin sulphuric acid is partly responsible for the elasticity of the cartilage, and it would therefore have a mechanical function. This subject needs to be developed further from physico-chemical aspects.

2. *The acidity.* The acid radicals could be regarded as activating certain enzyme systems and inhibiting others. Thus, for example, they could prevent the alkaline phosphatases from bringing about ossification. An alkali-binding factor might be discussed further.

All further propositions, however, are impeded by the fact that we do not know enough about the natural state of the chondroitin sulphuric acid, or about the manner in which it is linked to protein.

I believe that our previous views on the biology of cartilage are too static and strictly morphological. We seem to have been forgetful of the fact that 10-40 % of the dry weight of cartilage is made up of a polysaccharide-protein complex with very special structure and characteristic properties. We have

good reason to think that *chondroitin sulphuric acid occupies a central position in the biology of cartilage*, and plays an important part in its metabolism and function. If greater attention is paid to the study of this substance it should be possible to elucidate a large number of fundamental problems concerning the biological processes in cartilage.

A list of references will be found in J. Bone Jt. Surg. 1947, and 1948, where the paper was published in greater detail.

SUMMARY

The author discusses the chemistry of chondroitin sulphuric acid and its formation in the intercellular substance of cartilage, and describes our recent knowledge of its biology, from which he concludes that there are good reasons for believing that chondroitin sulphuric acid occupies a central position in the biology of cartilage.

RESUME

L'auteur parle de la chimie de la chondroïtine sulfurique et de sa présence dans la substance intercellulaire du cartilage et signale ce que nous savons actuellement de sa biologie, en relevant qu'il y a de bonnes raisons de croire que la chondroïtine sulfurique occupe une position centrale dans la biologie du cartilage.

ZUSAMMENFASSUNG

Verfasser behandelt die Chemie der Chondroitinschwefelsäure und ihr Vorkommen in der Interzellularsubstanz des Knorpels und gibt eine Darstellung unserer jetzigen Kenntnis ihrer Biologie. Er hebt hervor, dass gute Gründe für die Annahme bestehen, die Chondroitinschwefelsäure nehme eine zentrale Stellung in der Biologie des Knorpels ein.