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EXPERIMENTAL INVESTIGATIONS REGARDING THE AMOUNT OF MUCOPOLYSACCHARIDES IN THE TENDONS AFTER PROLONGED MUSCULAR STRAIN

By

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I. INTRODUCTION AND SURVEY OF LITERATURE

It is well known that the composition of a tendon, at the place of its attachment to the skeleton, differs from that of its other parts. The above-mentioned area consists of a so-called chondroid substance which, as the term indicates, offers certain similarities to cartilage. Instead of the typical flattened tendon cells, rows of round cells appear between the fibres of the tendons, surrounded by capsulas of a basophil substance, like cartilage cells in the proper sense of the term.

Considering that the intercartilaginous substance is known to abound in mucopolysaccharides, histologically characterized by the fact that certain stains produce a metachromatic colouring, and in histological works mostly described by the term "chromotropic substance", the question arises whether the tendons do not also contain such a substance, at least at their insertions. Apart from *Holmgren's* (1940) statement that the chromotropic substance is to be found in both elastic and collagen tendons in cows and (1949) in tendons in feti of mammals and human beings, this matter has been practically ignored. *Holmgren* does not define the localization in the tendon.

In the course of some preliminary studies of the chromotropic substance in the Achilles tendons of mice, rats, guinea-pigs, rabbits and hares fixed and stained according to the directions of *Holmgren* (1940) and *Sylvén* (1941), I noticed that the chromotropic substance is to be found, in varying, though small amounts in the connective tissue part of the tendon, while being plentiful in the chondroid part, particularly in the above-mentioned basophil substance about the round cartilaginoid cells.

In my opinion, the chondroid tissue, in the part of the tendon closest to the point of attachment, is likely to depend on special mechanical factors. The capacity of the supporting tissues to adapt themselves to changed mechanico-functional circumstances is well known, and the possibility of this observations applying also to the chondroid tissue of the tendon suggests itself. The particular factor I had in mind to study was, in the first place, the hyperfunction.

An experiment was performed with a small number of mice. They were exercised daily on a rolling carpet. Then, their Achilles tendons were compared with those of some control animals, with particular regard to the chromotropic substance. However, no distinct differences could be noticed.

The present investigation represents, partly, a renewed, more comprehensive experiment of a similar type, performed on guinea-pigs with a more intense and prolonged exercise. Since I had some reason to count with the possibility of a negative result also in this instance, as regards the morphologic changes, I combined these experiments with a similar one in which a quantitative chemical determination of the amount of the chromotropic substance was substituted for the histological investigation.

II. MATERIAL AND METHODS

The choice of guinea-pigs for these experiments depended on the following reasons. It is fairly easy to teach this species of animal to run on a rolling carpet. Further, the tendons of the foot of a guinea-pig are big enough to produce easily a sufficient amount of tendinous substance, for the purpose of a chemical analysis, from a relatively moderate number of animals. At the same time, they are not too big to prevent the production, within a reasonable space of time, of a complete section series of a tendon, such as the Achilles tendon, from a comparatively large number of animals.

All the 120 male guinea-pigs used are of the same strain. The weights vary from 491 to 241 Gm. They were arranged in a series according to weight and then, every second animal was collected into one group, the remaining ones into another. The first-mentioned group was exercised. The latter served as a control group.

The 60 animals belonging to the exercised group have had to run on a rolling carpet for altogether one hour a day; for a period of two months (excluding Sundays) this was divided into two half-hours with an interval of five minutes. The carpet has been kept at a constant speed, making the animals run 2265 metres a day. (During the

experiment 3 animals had to be removed from the control group and 10 from the exercised group owing to disease or injury).

At the termination of the experiment, 37 exercised animals and an equal number of controls were picked out for a chemical analysis of the tendons.

For this purpose, the Achilles tendon and the tendon of the musculus plantaris of both hind legs of each animal were dissected, to the right and the left, as well as, in the forelegs, the tendon of the musculus flexor digitorum sublimis, i.e. altogether six tendons in each animal. The dissection was performed by the same person on all the animals, exercised and controls, and particular care was taken that the procedure should be uniform in all instances. The musculature was removed from the tendons, and then they were placed in pure alcohol, those from the exercised animals and those from the controls in separate vessels. When the tendons had been fixed, the remaining hairs were cleared away. The weighing procedure in both groups was as follows: the tendons were taken out of the alcohol and vacuum-dried into a constant weight before being weighed, then replaced in their respective vessels. Afterwards, the chemical analysis took place.

At the end of the experiment, 13 animals remained in the exercised group and 20 in the control group, to be histologically examined. In each animal both Achilles tendons were taken out, together with the posterior part of the calcaneus and the skin covering the tendon. Fixation and staining were performed acc. *Sylvén* (1941). After fixation for 24 hours in 4 per cent lead acetate and for another 24 hours in 10 per cent formalin, the preparations were decalcified in 5 per cent formic acid, and washed with 95 per cent alcohol. Then, paraffin embedding and serial sectioning followed. The right tendon was cut lengthwise in a complete sagittal section, the left transversely. In the latter instance, every twentieth section was preserved for examination. The sections were 10 μ thick. The staining was made with a ½ per cent toluidine blue in 20 per cent alcohol for half an hour. Differentiation was performed in 95 per cent alcohol for 5 minutes and in absolute alcohol for 2 minutes.

III. THE HISTOLOGY OF THE ACHILLES TENDON IN THE NON-EXERCISED ANIMALS

Like the above-mentioned preliminary experiments on several species of animals, an examination of the present normal material, comprising 20 tendons cut lengthwise and another 20 cut transversely from an equal number of animals, shows that there is plenty of

chromotropic substance to be found in the particular part of the tendon that is situated in the proximity of the insertion at the calcaneus and characterized by a chondroid tissue. In addition, the same substance will be noted in smaller amounts also in the remaining portion of the tendon consisting of connective tissue only.

To direct the attention, to begin with, to the accumulation of chromotropic substance close to the insertion, a comparison with a hematoxylin-eosin stained preparation shows that the basophil capsulas, about the round cells that lie in rows between the tendon fibres, correspond to those with a strongly pronounced chromotropism. Similarly, the less marked basophilism in the intermediate tendon fibres, mentioned by *Schaffer* (1930), corresponds to a relatively moderate degree of chromotropism, decreasing in strength towards the muscular body, in the same way as the basophilism.

When a tendon in the examined animals is attached to cartilage, the basophil and chromotropic substances, respectively, continue directly in the intercartilaginous substance without disclosing any sharp contours. Still, the boundaries of the above-mentioned substances can be approximately localized by means of the adjacent free cartilaginous surface. At the place where the tendon fibres reach the latter, they are turned into a brilliant streak, of a green colour in the toluidine-stained preparations, continuing slightly into the cartilage.

In the tendons of all the numerous toluidine blue-stained preparations the same pictures, with certain variations in degree, are, on the whole, to be found. The rows of round cells with chromotropic capsulas, whose presence lend a cartilaginous character to this part of the tendon, may continue towards the muscular body. The capsulas may vary in thickness, the intensity of the chromotropism, in the parts of the tendinous fibre close to the tendinous insertion, may also vary and, like the rows of round cells, it may extend more or less distally from the insertion. Broadly speaking, there is a certain parallelism to be noted here. Wherever the rows of round cells extend far proximally in the tendon, the same will apply also to the chromotropism in the intermediate tendinous fibres. The extent of the distal part of the tendon, which contains fairly considerable amounts of chromotropic substance, equals about 2-3 tendinous diameters.

In the remaining parts of the tendon, no chromatic substance is to be found in the tendinous fibres, except in the portions closely contiguous to the muscular body. There, a faintly noticeable metachromasia can be traced in the fibres for a short distance.

Finally, it should be added that an often very pronounced metachromasia is observed in many places of the perimysium internum.

IV. THE HISTOLOGY OF THE ACHILLES TENDONS IN THE EXERCISED GUINEA-PIGS

The purpose of exercising a group of animals before the histologic examinations was, as already stated, to ascertain, as far as possible, whether the chromotropic substance is affected, in diffusion or amount in the tendon, when the latter has been subjected to an intense strain.

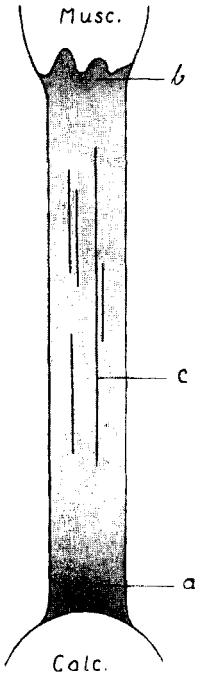

Fig 1.

A schematic representation of the distribution of the chromotropic substance in the Achilles tendon. Musc = the muscular body; calc. = the calcaneus; a = chromotropic substance in the proximity of the tendinous insertion; b = close to the muscular body; c = in the peritendineum internum.

As far as the diffusion is concerned, the chromotropic substance is to be found in the same place as in the unexercised tendons, viz. in a continuous area in the proximity of the tendinous insertion, close to the muscular boundary and, further, in the perimysium internum.

The position of the chromotropic substance in the area of the insertion conforms to that in the unexercised animals, i.e. it is to be found round the cells, as well as in the tendinous fibres. Also in the latter group of animals, certain individual variations are to be observed in the longitudinal diffusion within the area containing chromotropic

substance. When the two groups of animals are compared, with regard to the diffusion, no conspicuous difference appears. This applies also to the chromotropic substance found in other tendinous parts, i.e. close to the muscular body and the peritendineum internum. No doubt, the paper-weighing method would offer opportunities of studying the diffusion of the chromotropism more closely, and possibly, a difference in diffusion betwen the two groups of tendons would appear in that way. For practical reasons, I have been obliged to reject this very slow and complicated method.

The question whether the amount of chromotropic substance increases in the exercised Achilles tendons can, in my opinion, hardly be answered, at present, by means of any morphological examinations. This is a matter of a chemical substance that produces a certain colour reaction. To begin with, it is not definitely known whether the intensity of this reaction is directly proportionate to the amount of chromotropic substance. Even in the event of such knowledge being available, enormous difficulties would be met with in any attempts to secure a quantitative measure of the colour reaction and to determine simultaneously, as would be necessary, the local extent in the tendons of this reaction. I have refrained from all efforts in this direction, and substituted a quantitative chemical determination.

V. THE CHEMICAL DETERMINATION OF THE AMOUNT OF MUCOPOLYSACCHARIDES IN THE TENDONS

The quantitative determination has been performed at the Medicochemical Institution of Uppsala University, under the supervision of its Head, Professor G. Blix, by methods conceived by him.

From the extracts of the collected tendons from the exercised animals and the controls, the respective amounts of glucosamine (obtained from chondroitin-sulphuric acid and hyaluronic acid) were determined. Calculated on the dry substance of the tendons, the glucosamine constituted 186 mg per cent, for the exercised animals, and 137 mg per cent for the controls. The sulphur values were too small in these extracts to be at all reliable. For this reason it was not possible to distinguish betwen chondroitin-sulphuric acid and hyaluronic acid.

In the residua after the extraction, the total glucosamine and the ester sulphuric acid were determined. In the tendons of the exercised animals the glucosamine was found to amount to 97 mg per cent, in those of the controls to 105 mg per cent. The sulphur values proved

to be 15 mg per cent and 16 mg per cent, respectively. The ratio of glucosamine and sulphur has been interpreted as indicating that, in this instance, chondroitin-sulphuric acid is present practically exclusively.

When the contents of extracts and residua are added together, and counted as chondroitin-sulphuric acid, a calculation based on the tendrinous substance will give 0.73 per cent for the exercised animals and 0.62 per cent for the controls. Accordingly, the prolonged exertion produced an increase in the amount of mucopolysaccharides of 17.7 per cent.

A conspicuous difference appeared in the content of dry substance in both groups of tendons, the exercised animals containing 77 per cent and the others 60 per cent of dry substance. Considering that the two groups were treated in an identical manner, the difference suggests a higher swelling pressure in the colloids of the exercised tendons than in those of the controls.

VI. DISCUSSION

The most notable result of the present investigation is that a prolonged strain on the muscles of the lower extremities produces an increase in the mucopolysaccharides in certain tendons of the foot, ascertainable through a quantitative chemical analysis.

The increase, which amounts to 17.7 per cent, cannot be regarded as absolutely and irrefutably proved, considering the fact that tendons from two groups of animals, each comprising 37 specimens, have been examined and compared, that the extent of the individual variations is unknown and consequently also the mathematical distribution. On the other hand, the large number of tendons is calculated to eliminate this factor. There should, therefore, be sufficient justification, for stating that an increase surely occurs, though its degree is somewhat uncertain.

The histological examination of one of the exercised tendons (the Achilles tendon) shows that no distinct change can be demonstrated by the usual morphological methods. It may be objected that the explanation of this incongruity is, very conceivably, that the above-mentioned increase fails to appear in the Achilles tendon, while occurring in the two other chemically analysed tendons. Such a possibility seems, however, to be extremely unlikely, and there appears to be good reason for concluding that the chemical method is the only

one that can be applied to studies of the amount of the chromotropic substance.

As a matter of course, morphological studies of the chromotropic substance will be useful, provided the method is not pursued too far. Morphologically, it is easy to trace the particular tendinous parts that contain chromotropic substance, and by a suitable combination of the two methods it should, no doubt, be possible to carry the studies of the effects of a hyperfunction still further. The isolated lowest parts of the tendons could, e.g., be examined by themselves and the remainder subjected to a separate chemical determination, with a view to localizing the increase more closely. Such studies of the tendinous insertions seem to be indicated, inter alia, by the well known fact that an overexertion of a muscle, or group of muscles, in Man will often produce pains and soreness in precisely those places (e.g., humeral epicondylitis in factory workers). The question arises whether these pains might not be caused by a marked local increase in the amount of mucopolysaccharides, to the effect that the chondroid character of the tendon becomes still more pronounced. In this connection, it should be remembered that the above-mentioned chemical analysis suggest a bigger swelling pressure in the colloids of the exercised tendons than in those of the unexercised ones.

VII. SUMMARY

1. A metachromasia manifesting the presence of mucopolysaccharides appears in the Achilles tendons in guinea-pigs, being particularly marked in the chondroid part of the tendon situated nearest to the insertion, but noticeable also, though to a smaller extent, in the part immediately adjacent to the muscular body, as well as here and there in the perimysium internum.
2. A prolonged exercise did not produce any conspicuous morphological changes, while a chemical analysis showed that tendons of the feet of the exercised animals contained a larger amount of mucopolysaccharides, per weight unit of the total tendinous substance from a considerable number of animals, than tendons from unexercised animals.
3. Attention is drawn to the fact that the possibility of an increase in the amount of mucopolysaccharides and of the swelling pressure in the colloids of the tendons may conceivably explain the pains at the tendinous insertions in connection with an overexertion of the muscles.

RESUME

1. Une chromotropie marquant la présence de mucopolysaccharides apparaît dans le tendon d'Achille chez les cobayes, particulièrement prononcée dans la partie chondroïde du tendon la plus proche, mais aussi à un plus faible degré dans la partie la plus rapprochée du creux du muscle, de même qu'ici et là dans le perimysium interne.
2. Un entraînement de longue durée n'a pas provoqué de modifications morphologiques évidentes, mais un examen chimique a démontré que les tendons du pied provenant d'animaux entraînés contiennent une plus grande quantité de mucopolysaccharides par unité de poids de la totalité de la substance tendineuse d'un grand nombre d'animaux que ceux des animaux n'ayant pas d'entraînement.
3. Il est signalé qu'il est possible qu'une augmentation de la quantité des mucopolysaccharides et de la pression des colloïdes des tendons explique peut-être les douleurs ressenties dans les attaches tendineuses lorsqu'il y a surmenage des muscles.

ZUSAMMENFASSUNG

1. Eine Chromotropi, die das Vorhandensein von Mucopolysacchariden anzeigt, tritt in den Achillessehnen vom Meerscheinchen besonders stark in dem der Ansatzstelle zunächst gelegenen chondroiden Teil hervor, aber auch in geringerem Grade in dem dem Muskelbauch am nächsten liegenden Anteil und im Perimüsium. internum.
2. Ein langdauerndes Training hat niemals zuverlässige morphologische Veränderungen ergeben. Die chemische Untersuchung jedoch hat gezeigt, dass Fusssehnen der trainierten Tiere eine grössere Menge von Polysacchariden per Gewichtseinheit der gesammten Sehnensubstanz einer grossen Anzahl von Tieren enthalten als dies der Fall bei untrainierten Tieren ist.
3. Man weist auf die Möglichkeit hin, dass eine Zunahme der Menge der Polysaccharide und des Schwellungsdruckes der Sehnenkolloide die Schmerzen erklären könnten, die in den Sehnenansätzen nach Überanstrengung entstehen.

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