appears extracellularly, which is the precursor of both acid soluble and mature form[10]. A uniform decrease in the salt extractable collagen, which is generally accepted as the newly synthesized material, could be either due to decrease in the rate of synthesis with a constant turnover, or increase in the turnover rate with a constant rate of synthesis, or variations in the conversion rates of salt soluble to acid soluble collagen during aging. When the ratios of salt soluble to acid soluble, and acid soluble to insoluble collagen (Table) are compared, it is seen that there is accumulation of more mature or insoluble collagen which probably indicates the occurrence of cross linkages during the maturation of fibres. The quantitative variations in salt soluble, acid soluble and insoluble collagen probably may have physiological implications during aging. However, the accumulation of more mature form of collagen in muscle with advance in age is disadvantageous to the organism, since it affects the contractility and muscle mechanics, although it contributes strength and support to the tissue[10]. The present study is a preliminary report on the characterization of metabolism of collagen during aging.

Summary. The salt, acid and insoluble collagen fractions were estimated in red, white and cardiac muscles of 10-, 15- and 20-month-old albino rats. The total collagen level with reference to total proteins is more in red than in white and cardiac muscle. Accumulation of more of insoluble collagen and decrease in salt extractable collagen is seen in all three muscles with aging.

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[10] F. MAROTT SINEX, in *Advances in Gerontological Research* (Ed. L. BERNARD STREHLER; Academic Press, New York 1964), vol. 1, p. 165.

[11] Acknowledgment. We acknowledge our thanks to Dr. A. R. KASTURI BAI for the facilities and encouragement and to Dr. R. V. KRISHNAMOORTHY for valuable suggestions and C.S.I.R. for financial assistance.

Studies on Ageing of Collagen by Perchlorate Reactions

Tendon fibres are a biological object of nearly pure collagen which is of plasma origin and has well defined age changes. Isometric tension of tendon fibres increases with the age of the animal. We studied different factors which influence these age changes[1-3]. In the following experiments, we use sodium perchlorate to stimulate isolated collagen-tendon-fibres to isometric tension production under different conditions, such as age, influence of aldehyde, etc. and changes of the fibres weight.

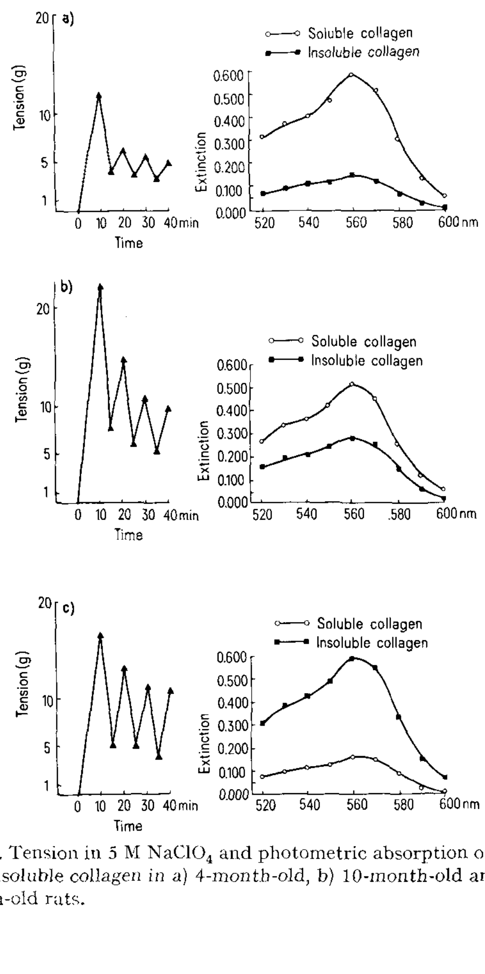

Fig. 1. Tension in 5 M $NaClO_4$ and photometric absorption of soluble and insoluble collagen in a) 4-month-old, b) 10-month-old and c) 25-month-old rats.

Methods. Isometric tension is produced by immersing tendon fibres in 5 *M* $NaClO_4$ solution (signed PC). Tail tendons of rats are used which are 50 mm long, have a diameter of $\pm$ 0.15 mm and a weight of $\pm$ 4–5 mg. They can be fixed in an electric tensimeter at low tension and immersed in different solutions. Tension changes at constant length are measured. They are analyzed and their content of soluble and insoluble collagen measured by photometric absorption (NEUMANN and LOGAN[4]). We used an Elco photometer and registered between 520–620 nm; 562 nm is used as the value for hydroxyprolin (i.e. collagen) content. The exchange of different solutions, such as of physiological NaCl (0.85%), or solutions of formaldehyde and other substances, is described in the text.

Experiments. Collagen-tendon-fibres were immersed in 5 *M* $NaClO_4$. An increase of tension starts immediately and becomes maximal in about 1 min. It then relaxes again. Fibres of young and old animals behave differently; their tension decreases in young in a shorter time than in old animals tendon. Continuous immersion in PC will result after a few minutes in a definitive disappearance of tension capacity in the relaxed state. If, however, the fibre, after it has been immersed in PC and produced maximal tension, is immediately transferred into physiological NaCl (0.85%), then the tension decreases again to about the original value and tension and relaxation can now be repeated several times. We generally used 4 tension tests for each experiment. Figures 1 a–c show

[1] A. MEYER and F. VERZÁR, Gerontologia *3*, 184 (1959).
[2] F. VERZÁR, Int. Rev. connect. Tissue Res. *2*, 245 (1964).
[3] F. VERZÁR, E. STRITTMATTER-ACKERSCHOTT and P. MOSER, Gerontologia *19*, 129 (1973).
[4] R. E. NAUMANN and M. A. LOGAN, J. biol. Chem. *184*, 299 (1950).

tension of 4-, 10- and 25-month-old animals tendon fibres by immersion in 5 *M* PC and then their relaxation in physiological NaCl. Tension and relaxation are greater in old animals.

In the same animal's tendon-fibres, we estimated the quantity of soluble and insoluble collagen, after the 4 tests in 5 *M* PC. Figures a–c show the photometric absorption with fibres from 4-, 10- and 25-month-old animals.

In Figure 1a the tendon of a young (4 month) animal contains about 4 times more soluble than insoluble hydroxyprolin (i.e. collagen), 590 against 150 scale parts. In Figure 1b the fibre of a 10-month-old subject showes 510 soluble and 280 insoluble scale parts, and in Figure 1c in a fibre of a 25-month-old animal shows mainly insoluble collagen (about 600 insoluble and 150 soluble scale parts). Thus, after treatment with PC tendon fibres collagen shows an increase of insolubility as a sign of ageing.

The registration of tension of the same age tendon fibres in Figures 1a–c should be compared with these solubility values. The 25-month-old animal, with highest insolubility of collagen, has also the highest tension values. Several similar experiments were performed.

It was then found that if formaldehyde was added to the tendon fibre in concentration of 0.35% up till 3.5%, the tension which is produced in perchlorate becomes much increased. Aldehyde by itself has no influence on the fibres tension. Only if after previous aldehyde the fibre is transferred to the 5 *M* $NaClO_4$ solution, does an increased tension occur, as Figures 2, a–c show. Not only is the tension increased, but tension and relaxation can be repeated now many times without increasing the aldehyde.

On the 4-month-old animal in Figure 2a, the tension is larger and continuous. It is completely different to the equally 4-month-old fibres tension in Figure 1a, while the young animals' fibre showed a great quantity of soluble collagen, in Figure 2a, with another 4 month-old fibre after aldehyde treatment, it is very small. In the 10-month-old animal in Figure 2b as also in Figure 2c, in a 22-month-old-fibre, there is no soluble collagen present.

Only by decreasing the aldehyde concentration to 0.035% soluble collagen appeared again. Obviously this concentration seemes to be on the lower limit of aldehyde activity on collagen.

We studied the influence of a few other substances also. Methionin, the amino acid present in collagen, which contains S groups, inhibits in 1% solution the production of tension by perchlorate. It also diminishes both soluble and insoluble collagen content, as Figure 3 showes.

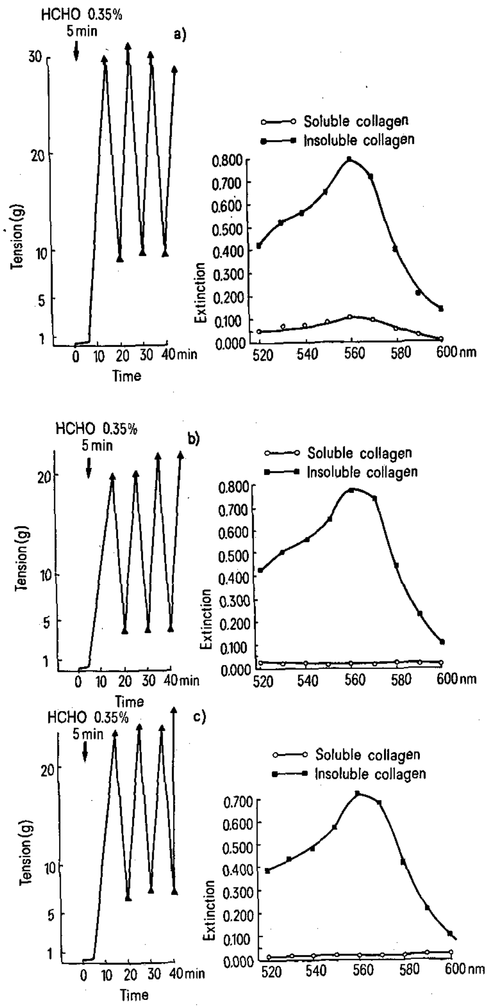

Fig. 2. Tension in $NaClO_4$ with 0.35% formaledehyde and photometric absorption of soluble and insoluble collagen in a) 4-month-old, b) 10-month-old and c) 22-month-old rats.

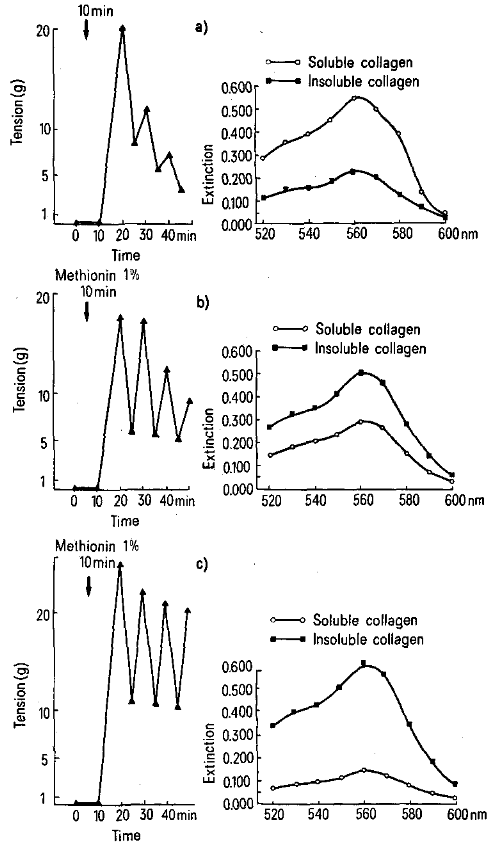

Fig. 3. Tension in $NaClO_4$ with 1% methionin and photometric absorption of soluble and insoluble collagen in a) 4-month-old, b) 10-month-old and c) 28-month-old rats.

It was then observed that if a tendon-fibre is immersed in 5 *M* $NaClO_4$ and the fibre produces isometric tension, at the same time the weight of the fibre itself increases. As an example: a 6-month-old animals tendon fibre produced 17 g isometric tension and the weight of the fibre increased from 5 to 28 mg. A 28-month-old animals tendon fibre, under the same conditions, produced 27 g isometric tension and the weight of the fibre increased from 5 to 32 mg. The tension relaxes to its original value in the 6-month-old animal in 50 min, while in the 28-month-old animal in about 130 min.

As Figure 4 showes the weight of the fibre remains increased in such experiments. Weight estimations in short periods are only possible by interrupting tension each time. Generally it was sufficient to test the weight before and on the end of the experiment. All these observations were repeated several times.

The increase of weight of the fibre in PC means that PC fluid enters the fibre which is "swelling" from its natural isotony to 5 *M* concentration, like the surrounding solution. If a fibre is left after relaxation in PC for some minutes, it cannot produce tension again. Thus it seems that in the PC solution the fibre has somehow altered its structure.

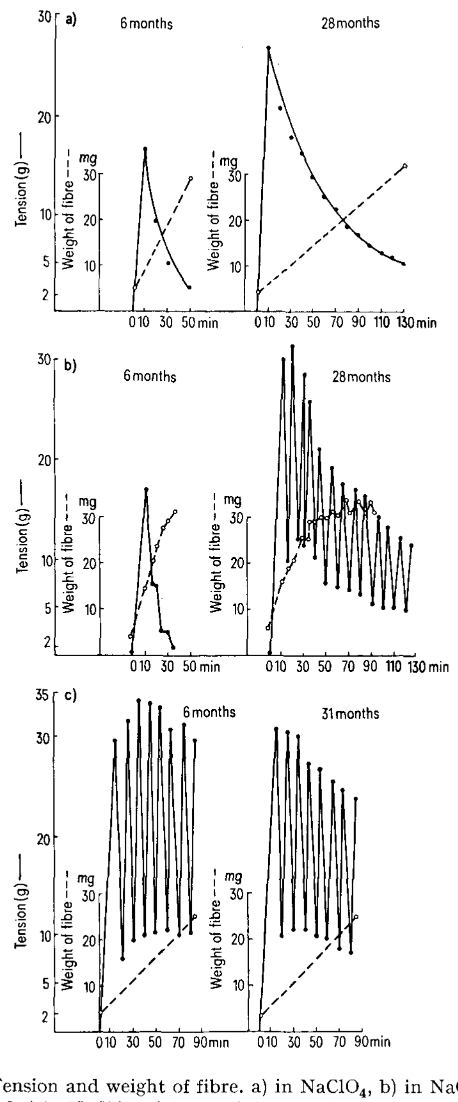

Fig. 4. Tension and weight of fibre. a) in $NaClO_4$, b) in $NaClO_4$ and NaCl, and c) in $NaClO_4$ with 0.35% formaldehyde.

In contrast to this it is possible to reverse relaxation into tension again if the relaxation was made in physiological NaCl (0.85%). Tensions and relaxations can be produced with the same fibre continuously by such repeating. The weight of the fibre does not change during such repeats.

Similar experiments can be repeated if first aldehyde was added. The tension in PC then increased. It should be underlined that if 0.35% formaldehyde is added only once to the fibre before PC, the tension will remain increased if repeated for a long time.

Summarizing the above experiments, we found that sodiumperchlorate stimulates tendon fibres to increased tension. This "chemical stimulation" can be demonstrated even with 2 *M* PC, and is maximal with 5 *M* PC. This reaction is reversible by exchanging PC with physiological NaCl, in which there is relaxation. Tension can be increased by formaldehyde, if this is given before the perchlorate. It is diminished by methionin and other cystein producing substances[3]. This increase of collagen tension, both in force and duration of action by aldehyde seemes to show that the tension reaction destroys some chemical factor of collagen, which can be restored and increased by the addition of aldehyde. Actually even with low aldehyde concentration, 5 *M* $NaClO_4$ gives long and strong tension reactions.

Discussion. The problem how tension is produced in the collagen fibre has been discussed by several authors. BAILEY and ROBIN[5–8] in their review of 1973 explain tension production in the following way: "The stabilization of the collagen fibre is based on a system of crosslinks ... derived from lysine and hydroxyproline. The fibres contain labile aldimines as intermolecular crosslinks, but during maturation these intermediate crosslinks are stabilized to an as yet unidentifed non-reducible crosslink". We agree with their opinion that there is no convincing chemical evidence on the changes in the crosslinking, but we add that the tension measurements show a continuous increase of tension even after maturation in old age.

HATEFI and HANSTEIN[9,10] have discussed the problem in a different way: "Lipid oxydation in submitochondrial particles and microsomes induced by chaotropic agents by oxydation by molecular oxygen of unsaturated fatty acid residues of phospholipids ... initiated by free radicals ... as malondialdehyde"[10] (p. 73).

In a second paper HANSTEIN and HATEFI[11] feel that "stability of membrances and intergrated enzyme systems may be directly related to the degree of ordered structure in the surrounding water" (p. 95). They discuss the possibility that some factor may influence the structure of membrane permeability and the influence of diffusing substances[12,13].

[5] A. J. BAILEY, Gerontologia *15*, 65 (1969).
[6] A. J. BAILEY and M. S. SHIMIKOMAKI, FEBS Lett. *16*, 86 (1971).
[7] A. J. BAILEY, Front. Matrix Biol. *1*, 130 (1973).
[8] S. P. ROBIN, M. SHIMOKOMAKI and A. J. BAILEY, Biochem. J. *131*, 771 (1973).
[9] Y. HATEFI and W. G. HANSTEIN, Science *62*, 1129 (1969).
[10] Y. HATEFI and W. G. HANSTEIN, Arch. Biochem. Biophys. *138*, 73 (1970).
[11] W. G. HANSTEIN and Y. HATEFI, Arch. Biochem. Biophys. *138*, 87 (1970).
[12] K. A. DAVIS and A. HATEFI, Arch. Biochem. Biophys. *149*, 505 (1972).
[13] W. G. HANSTEIN, K. A. DAVIS and Y. HATEFI, Arch. Biochem. Biophys. *147*, 534 (1971).

NÉMETHY and SCHERAGA[14,15] have discussed changes of water structure. They referred to the production of "clusters" which may influence permeability in the fibre etc. We may add that the fact that sodiumperchlorate has an intensive influence on collagen tension production, while $NaClO_3$ is completely inactive, may lead one to question the role of oxydation of collagen by perchlorate.

If the collagen fibre is transferred from 0.85% NaCl to the 5 *M* perchlorate, the osmotic pressure difference will drive perchlorate into the fibre and result in the observed increase of fibre weight, as the experiments show.

It may be worthwhile to follow the role of the aldehyde activity on collagen. One action is that aldehyde diminishes the solubility of collagen and increases its insolubility remarkably. It prolongs and increases the mechanical tension also. Young fibres have mainly soluble collagen while in old animals' fibres only insoluble collagen is present.

While former studies showed that the tension of collagen fibres increases with the animals' age, it is now found that studies with $NaClO_4$ and aldehyde present farther possibilities in the analysis of tension-producing factors. This mechanism is not explained in our experiments, but one is reminded that it may have a relation to the observation that in ageing, under biological conditions, tension changes appear. It remains to follow up with research along these lines.

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Institute of experimental Gerontology, Nonnenweg 7, CH-4055 Basel (Switzerland),
11 June 1975.

[14] S. NÉMETHY and H. SCHERAGA, J. chem Phys. *36*, 3382 (1962).
[15] G. NÉMETHY and H. SCHERAGA, J. chem. Phys. *36*, 3401 (1962).

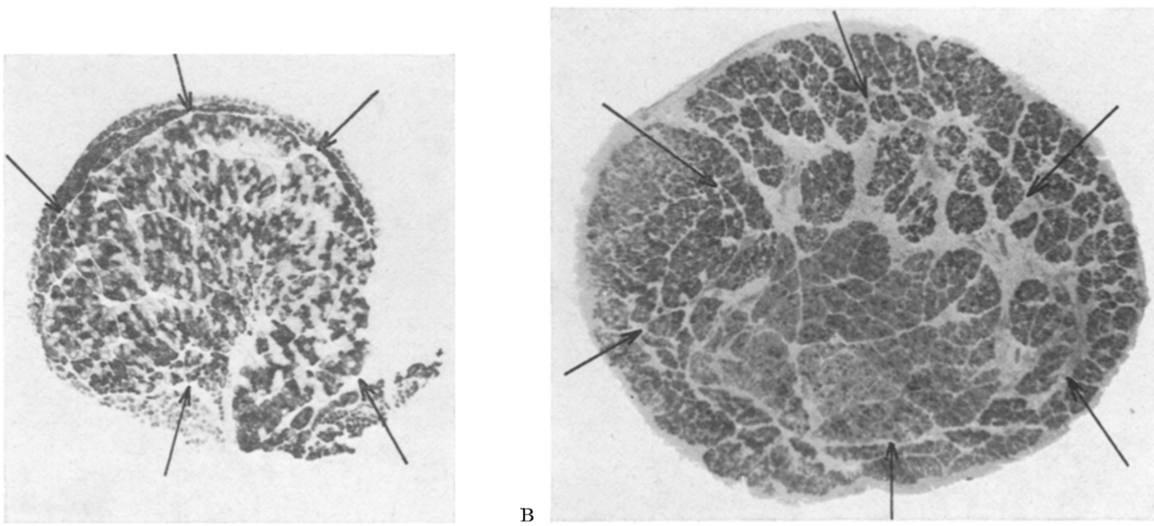
Hypertrophy and Atrophy of Mammalian Extraocular Muscle Fibres Following Denervation

There are at least six different types of muscle fibres in mammalian extraocular muscles[1-3]. Some of them have single neuromuscular junctions (phasic muscle fibres), others have multiple innervation by diffuse en grappe nerve terminations (slow tonic muscle fibres). Reactions of muscle fibres to denervation may be caused by the lack of neurotrophic influence from their motor innervation and by passive stretch, which influences the inactive muscle during activity of its antagonists. According to data available on reactions to denervation of various vertebrate muscles, different responses of the single muscle fibre types composing extraocular muscles are to be expected. Avian slow tonic muscle fibres show a long-lasting hypertrophy after denervation, especially if an additional stretch is given (reviewed by[4]). Common skeletal muscles (phasic) are known to react to deprivation of their motor innervation by atrophy[5], the phasic muscle fibres of the denervated hemidiaphragm, however nndergo a transient hypertrophy[4,6].

So far no reports were published about calibre changes of muscle fibres of extraocular muscles following denervation. It will be shown that the reactions of the different types of fibres found in extraocular muscles were as varied as was to be expected, considering the results mentioned above.

Material and methods. After anesthetizing adult rabbits with barbiturates, their right orbitae were opened. The inferior oblique muscle was denervated by removing a 3–5 mm segment of the nerve bundle innervating the muscle (belonging to N. oculomotorius). The results of denervating this single muscle were assessed histochemically 7, 21, 28, 34, 46, 55 and 83 days after denervation. At these times, the rabbits were sacrificed and the inferior oblique muscles of both sides were removed. The de-

[1] G. ASMUSSEN, A. KIESSLING and F. WOHLRAB, Acta anat. *79*, 526 (1971).
[2] R. MAYR, Tissue Cell *3*, 433 (1971).
[3] D. W. HARKER, Invest. ophthal. *11*, 956 (1972).
[4] D. M. STEWART, O. M. SOLA and A. W. MARTIN, Z. vergl. Physiol. *76*, 146 (1972).
[5] E. GUTMANN, *The Denervated Muscle* (Publishing House of the Czechoslovak Academy of Sciences, Prague 1962).
[6] H. YELLIN, Expl. Neurol. *42*, 412 (1974).



A B

Fig. 1. M. obliquus inferior, rabbit, SDH-activity, cross section. × 20. A) control. B) 55 days after nerve section. The border between superficial (orbital) and central (global) layers are marked by arrows.