

zyten^{10,11} oder eine Erhöhung bzw. Erniedrigung des Hämatokrits von Bedeutung⁶. Zu einer umfassenden Charakterisierung der Fließeigenschaft gehören daher auch Messungen der entsprechenden Einzelparameter⁹. Für Routinemessungen sowie als Suchmethode über die eventuelle Bedeutung der Viskosität haben sich die Messungen der Plasma- und Vollblutviskosität bewährt¹⁰.

Zur Messung der Plasmaviskosität sind seit langem Kapillarrisikometer ausreichend geeignet. Die Vollblutviskosität bedarf jedoch wegen des strukturviskosen Verhaltens des Blutes weiterer und anderer Messvoraussetzungen^{9,12}. Wesentlich ist hierbei die Messung bei unterschiedlichen Schergraden. Besonders interessant

für medizinische Aussagen sind die Schergrade unter 100 sec^{-1} , da etwa in diesem Bereich das Blut von einem Newtonschen Fließverhalten in ein plastisches bzw. bei extrem niedrigen Schergraden in ein pseudoplastisches Fließverhalten übergeht^{9,12}. Ideal für die Messung der Vollblutviskosität wäre daher ein Gerät, das bei extrem niedrigen Schergraden, d.h. unter 50 sec^{-1} , optimale Messbedingungen aufweist. Für Geräte, die mit dem Platte-Kegel-System arbeiten und damit preislich erschwinglich sind, ist dies nach unseren Untersuchungen nur beschränkt möglich und verlangt zumindest Erfahrung in der Messtechnik.

Die Ergebnisse beim Kapillarrisikometer zeigen wegen der grössenordnungsmässig gleichen Abweichung vom Eichwert bei drei unterschiedlichen Kapillaren, dass der limitierende Faktor dieser Messkette wahrscheinlich in der Temperatureinstellung und eventuellen Temperaturkonstanz liegt. Die Bedeutung der Temperatur auf die Viskosität zeigen die weiteren Untersuchungen. Hierbei führen geringe Temperaturerhöhungen nicht nur zu einer Senkung der Plasmaviskosität, sondern darüber hinaus muss ein direkter Einfluss auf die Erythrozyten angenommen werden, da das Vollblut prozentual eine fast doppelt so grosse Verminderung der Viskosität gegenüber dem Plasma zeigt. Nur durch zusätzliche Messungen der Aggregation und Flexibilität könnte dieser Effekt näher differenziert werden.

Ohne Einfluss auf die Fließeigenschaft bleibt dagegen eine Verminderung der Thrombozyten, obwohl damit nicht ausgeschlossen ist, dass extrem hohe Thrombozytenzahlen, wie bei der Thrombophilie, doch eine Bedeutung auf die Vollblutviskosität haben könnten.

Summary. The capillary viscosimeter (Viscotimer) tested with different Ubbelohde-viscosimeters is suitable for measurements of plasmaviscosity; the limiting factor of the measuring method is the exact adjustment of the temperature. When testing the plate-cone-viscosimeter (Wells-Brookfield Microviscosimeter), we achieved scientifically adequate results only when using shear rates 230, 115 and 46 sec^{-1} . An influence of different thrombocyte-concentrations on the viscosity of whole blood and plasma could not be detected. The flow capacity of whole blood and plasma is distinctly dependent on the temperature.

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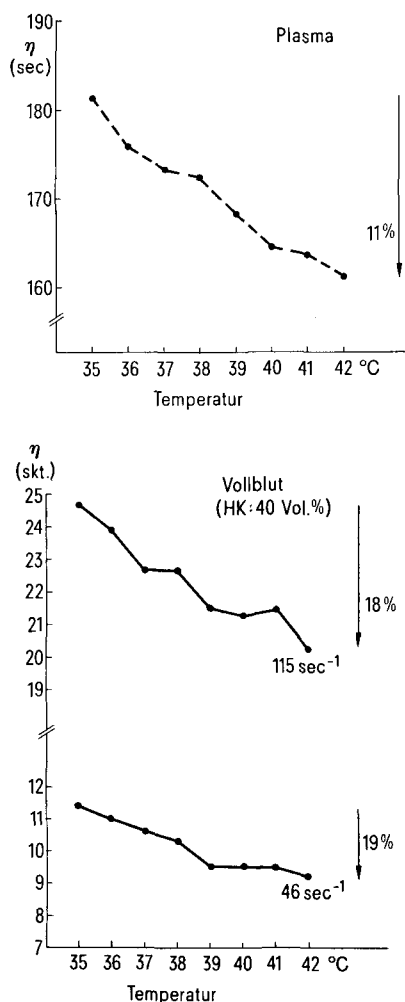


Fig. 2, a + b. Verhalten der Vollblut- und Plasmaviskosität bei physiologischen Temperaturänderungen.

Collagen in Aging Muscles

Collagen of mammals accumulates rapidly during growth in organs rich in connective tissue at a rate exceeding that of some other proteins¹. According to FISHER and RAMSEY², HELANDER³, and GUTH⁴, there is a relative increase in collagenous proteins and a decrease in myosin and total proteins in muscular atrophy of various types. However, the different fractions of collagen and their quantitative changes during aging with reference

to slow, fast, and cardiac muscles in the rat have not been reported so far, and hence the present study.

¹ D. A. LOWTHER, *Int. Rev. connect. Tissue Res.* 1, 63 (1963).

² E. FISHER and V. W. RAMSEY, *Am. J. Physiol.* 143, 571 (1946).

³ E. HELANDER, *Nature Lond.* 182, 1035 (1958).

⁴ L. GUTH, *Neurosci. Res. Progr. Bull.* 7, 1 (1969).

Materials and methods. Albino rats of wistar strain aged 10, 15 and 20 months, were maintained at $28 \pm 2^\circ\text{C}$ on a commercial diet. The animals were decapitated; the red, white and cardiac muscles were removed and weighed immediately. The water and protein content⁵ were estimated. Tissues from 2 animals were pooled for each estimation. The salt soluble, acid soluble, and insoluble collagen was extracted and purified according to JACKSON⁶, and hydrolyzed in 6 N HCl for 3 h at 50 ψ . The amount of hydroxyproline present in each fraction was then estimated by the colour reaction of STEGMANN as modified by WOESSNER⁷. The tissue somatic index⁸ ($\frac{\text{wet wt. of the particular muscle}}{\text{wet wt. of the animal}} \times 100$) was calculated.

Results. A uniform decrease, in tissue somatic index (Figure 1) water content and total proteins (Figure 2) is

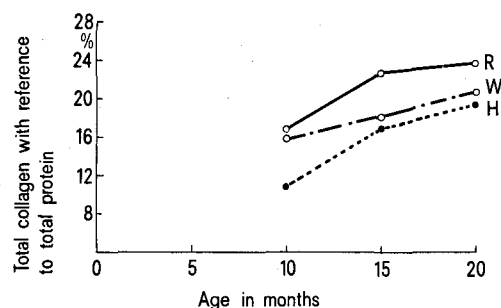
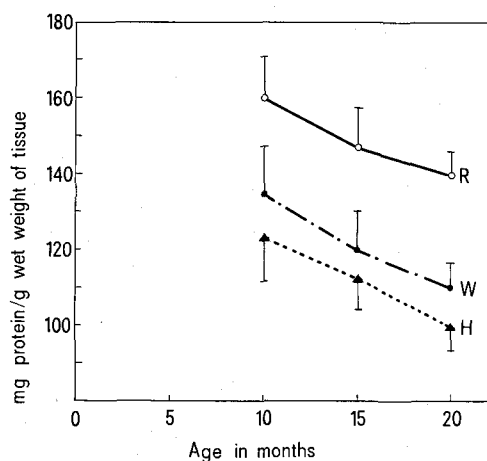
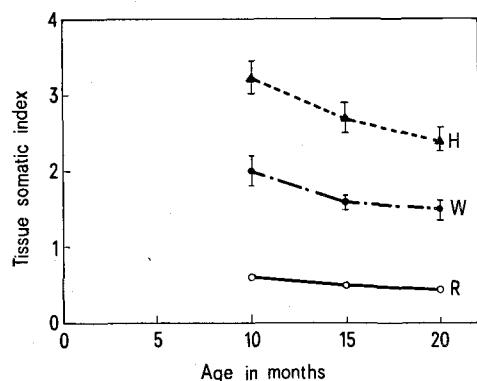


Fig. 1-3. Tissue somatic index (Figure 1), total protein content (Figure 2), and percentage total collagen with reference to total proteins (Figure 3) in red, white and cardiac muscles of 10-, 15- and 20-month-old albino rats. R, red (soleus); W, white (extensor digitorum longus); H, cardiac muscle.

The ratios of salt soluble to acid soluble and acid soluble to insoluble collagen in aging muscles of rat

Ratio	Age in months		
	10	15	20
Salt soluble			
Acid soluble	Red 0.4748	0.3890	0.3174
	White 0.2866	0.1512	0.1503
	Heart 0.4039	0.2536	0.1656
Acid soluble			
Insoluble	Red 0.8807	0.8211	0.6086
	White 0.7610	0.7987	0.6109
	Heart 0.7968	0.5297	0.6432

Each value represents the average of 4 observations in case of 10 and 20 months and 2 observations in case of 15 months.

seen with advance in age in all the 3 muscles. However, when compared to heart and white muscles, the red muscle contains more proteins (Figure 2) and collagen (Figure 3). The total collagen level expressed as percent collagen with reference to total proteins increases in all the 3 muscles with advance in age, but the rate of increase varies with the type of muscle (Figure 3), whereas salt soluble collagen decreases progressively in all 3 muscles in contrast to acid soluble and insoluble collagen (Figure 4).

Discussion. The decrease in the tissue somatic index of the muscles probably indicate the progressive senile muscular atrophy with increase in age. The present result, of increase in total collagen content in the heart muscle, supports the view of SOBEL et al.⁹. The salt soluble collagen seems to be the form in which collagen

⁵ E. LAYNE, in *Methods in Enzymology* (Eds. S. P. COLOWICK and N. O. KAPLAN; Academic Press, New York 1957), vol. 3, p. 454.

⁶ D. S. JACKSON, in *Methods of Biochemical Analysis* (Ed. D. GLICK; Interscience Publishers, New York, London, Sydney 1967), vol. 15.

⁷ J. F. WOESSNER, *Arch. Biochem.* 93, 440 (1961).

⁸ K. SATYANARAYANA, V. R. SELVARAJAN, V. CHANDRASEKHARAN, R. RAMAMURTHI and K. S. SWAMI, *Curr. Sci.* 44, 127 (1975).

⁹ H. SOBEL, H. THOMAS and R. MASSERMAN, *Proc. Soc. exp. Biol. Med.* 116, 918 (1964).

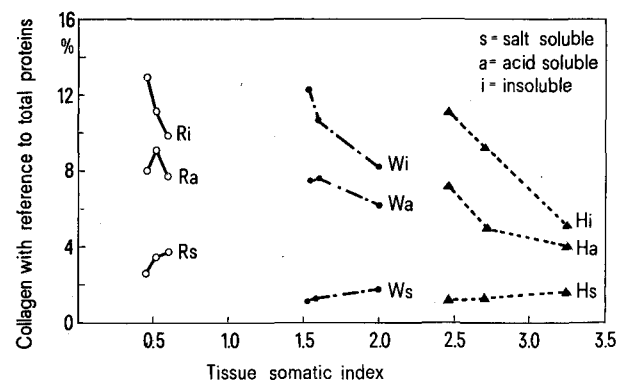


Fig. 4. Tissue somatic index versus percentage salt soluble, acid soluble and insoluble collagen with reference to total proteins in red, white and cardiac muscles of albino rats.

appears extracellularly, which is the precursor of both acid soluble and mature form¹⁰. 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 tissue¹⁰. 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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Bangalore 560001 (India), 21 March 1975.

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

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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 changes¹⁻³. 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.

Methods. Isometric tension is produced by immersing tendon fibres in 5 M NaClO₄ solution (signed PC). Tail tendons of rats are used which are 50 mm long, have a diameter of ± 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 LOGAN⁴). 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 NaClO₄. 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

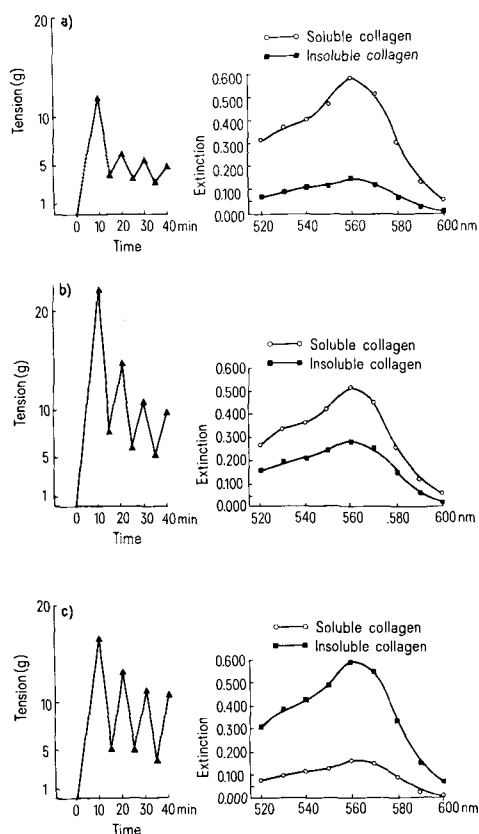


Fig. 1. Tension in 5 M NaClO₄ and photometric absorption of soluble and insoluble collagen in a) 4-month-old, b) 10-month-old and c) 25-month-old rats.

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).