

änderung der Nettoladung begleiteten Änderung der Moleküldimensionen ändern sich weitere Parameter wie zum Beispiel Viskosität und optische Drehung. Ein bekanntes Beispiel hierfür ist die N-F-Transformation des Albumins⁹. Das Albumin ist jedoch hinsichtlich seiner Struktur noch wesentlich stabiler als das Präalbumin, denn es wird durch zahlreiche Disulfidbindungen stabilisiert, während Präalbumin kein Cystein und somit keine Schwefelbrücken enthält. Albumin liefert nach unseren Befunden bei der Kristallisation mit Ammoniumsulfat im pH-Bereich von 4,6–7,7 immer die gleiche Kristallform. Schliesslich könnte die Anlagerung von Ionen des Fällungsmittels eine Rolle spielen. Sie sollte sich beim isoelektrischen Punkt (pH 4,7¹⁰) weniger bemerkbar machen, als bei pH-Werten, bei denen das Protein eine beträchtliche negative Ladung aufweist (pH 6,5 und 7,4). Eine pH-Abhängigkeit der Kristallform von Proteinen wurde schon früher von KING et al.¹¹ im Falle der Ribonuklease und von CZOK und BÜCHER¹² bei Enolase und Triose-Phosphat-Isomerase beobachtet.

Die erhaltenen Präalbumin-Kristalle waren auch nach mehrwöchigem Stehen stabil und lagerten sich nicht in andere Formen um. Sie besaßen die für Proteinkristalle wie zum Beispiel Hämoglobin charakteristische weiche Konsistenz, so dass sie bei mikroskopischer Betrachtung schon vom Deckgläschen zerdrückt wurden. Zum Nachweis ihrer Proteinatur wurden die Kristalle durch Abzentrifugieren von der Mutterlauge getrennt. Abguss und Rückstand wurden mit der Biuret-Reaktion sowie mit

der Extinktion bei 280 nm ($E_{1\%}^{1\text{cm}} = 13,2$) auf ihren Eiweissgehalt geprüft. Bei schneller Kristallisation (relativ kleine Kristalle) waren im allgemeinen 80–90% des Proteins kristallisiert. Bei langsamer Kristallisation waren nur etwa 60–70% des Proteins an der Kristallisation beteiligt.

Summary. The thyroxine binding prealbumin was crystallized by ammonium sulphate, sodium sulphate, magnesium sulphate, sodium phosphate and potassium phosphate. The form of the orthorhombic crystals was dependent on pH.

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*Behringwerke AG, Marburg, Lahn (Deutschland),
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⁹ J. F. FOSTER, in *Plasma Albumin* (Ed., F. W. PUTNAM; Academic Press, New York-London 1960).

¹⁰ R. GOT, R. BOURRILLON und J. MICHON, *Protides of the Biological Fluids* (Elsevier, Amsterdam 1962), p. 125.

¹¹ M. V. KING, B. S. MAGDOFF, M. B. ADELMAN und D. HARKER, *Acta crystallogr.* 9, 460 (1956).

¹² R. CZOK und TH. BÜCHER, *Adv. Protein Chem.* 15, 335 (1960).

¹³ Für Diskussionen über die Form der Kristalle sind wir Herrn Prof. Dr. E. HELLNER und Herrn Dr. R. ALLMANN vom Mineralogischen Institut der Universität Marburg zu Dank verpflichtet.

The Length-Tension Diagram of Single Muscle Fibres of the Crayfish

As shown by RAMSEY and STREET¹ on single twitch fibres of the frog, the tension developed in a maintained tetanus is a maximum at a definite length of the fibre and decreases with stretching or shortening of the fibre. The shape of the length-tension diagram is related very closely to the amount of overlap between the thick and thin filaments².

The length-tension diagram of isolated muscle fibres of the crayfish (Figure) differs from that of the frog muscle fibres by a steeper fall in tension after stretching the fibre beyond its optimal length. The diagram was constructed from the records of potassium contractures evoked by sudden application of solutions containing 174 mM K and 7.8 mM Cl. The potassium contracture represents practically the only available way of obtaining maximal tension in crayfish muscle fibres in view of the multiple innervation and the local character of membrane responses as well as of the accompanying contractions³. Single fibres were dissected from the extensor carpopoditi muscle of the crayfish (*Astacus fluviatilis*) and the tension was recorded by the RCA 5734 transducer⁴.

Crayfish muscle fibres develop maximal tension when stretched to 1.25 l_0 , where l_0 is defined as the length at which the fibre is just held taut horizontally in van Harreveld solution (5.4 mM K, 250 mM Cl). At the optimal length the sarcomere length was $10.5 \pm 0.3 \mu$ and the length of the A band was $3.95 \pm 0.8 \mu$. The sarcomere length was directly proportional to the amount of extension, the length of the A band remaining constant. It is

thus simple to transform the abscissa in units of initial length to the abscissa in microns of sarcomere length. The length of the sarcomere was measured from microphotographs made with the use of a double-objective microscope constructed according to the principle of LAU⁵.

When the fibre was stretched beyond 1.35 l_0 , there was an almost linear decrease in tension. The explanation is that the overlap between the thick and thin filaments decreases in this range of extension. If we take into account the values of the sarcomere length as well as the length of the A band measured at the optimal length (= 1.25 l_0), we would expect the tension to fall to zero values at the sarcomere length $10.50 + 3.95 = 14.45 \mu$, which corresponds to the extension of the fibre to 1.72 l_0 . At this length the thick and thin filaments ought to be just 'out of mesh' (inset diagram on the right). The fact that fibres are still capable of generating tension at this length can be explained by assuming that tension is generated in those parts of the fibre close to the insertions, where filaments still overlap, while central parts of the fibre are already 'out of mesh'⁶. The value of the residual

¹ R. W. RAMSEY and S. F. STREET, *J. cell. comp. Physiol.* 15, 11 (1940).

² A. M. GORDON, A. F. HUXLEY, and F. J. JULIAN, *J. Physiol.* 171, 28 P (1964).

³ D. ZACHAROVÁ and J. ZACHAR, *Physiologia bohemoslov.* 14, 401 (1965).

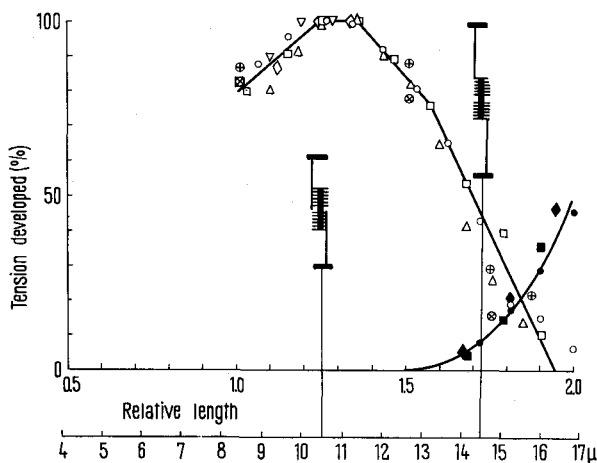
⁴ J. ZACHAR, D. ZACHAROVÁ, and M. HENČEK, *Physiologia bohemoslov.* 13, 117 (1964).

⁵ F. LAU, *Feingeräte-Tech.* 9, 112 (1960).

⁶ A. F. HUXLEY and L. D. PEACHEY, *J. Physiol.* 156, 150 (1961).

tension is close to that found on single twitch fibres of the frog⁷ in tetani where the tendons were held similarly to the present case. A better fit could be expected if the influence of shortening of the fibre at the ends on the length-tension diagram was avoided^{2,8}.

A steeper fall in tension after stretching the fibre beyond its optimal length in the crayfish as compared with the frog can be ascribed to a lower A/I quotient in the crayfish than in the frog. The overlap between the thick and the thin filaments is then expected to cease at lower relative extension of the fibre in the crayfish than in the frog.



The length-tension diagram of 8 isolated muscle fibres of the crayfish (*Astacus fluviatilis*). Hollow symbols: maximal tension developed during a potassium contracture (174 mM K, 7.8 mM Cl), which was relaxed by reintroducing the crayfish saline (5.4 mM K, 250 mM Cl). Symbols with crosses refer to fibres relaxed spontaneously. Full symbols: the resting tension about 10 min after slowly stretching the fibre. Both the active and passive tension are given in relative units taking the amplitude of contracture at $1.25 l_0$ as 100%. The lines through the experimental points were drawn by eye. Upper horizontal scale: the length of fibres in fractions of the slack length (= 1.0). Lower horizontal scale: the sarcomere length. The diagrams indicate the assumed degree of overlap between the thick and the thin filaments.

As the sarcomere length of crayfish muscle fibres is five times greater than that of twitch muscle fibres of the frog, one would expect from the sliding theory of contraction (the other factors controlling the tension⁹ being constant) a five times higher tension in crayfish muscle fibres than in frog muscle fibres. The maximal tension at the optimal length is, however, only about twice (8.2 kg/cm²) as high in the crayfish as in the frog (about 4 kg/cm²)^{1,10}. The explanation follows from the comparison of the A/I quotients in these fibres. The A/I quotient in the crayfish (0.38) is half that found² in the frog (0.76). It can then be assumed that the number of those sites per cm³ where the tension is generated, is in crayfish muscle fibres also half that present in frog fibres. A crayfish muscle fibre should then exert tension only 2.5 times greater than a frog fibre, which is in good agreement with the measured values of maximal tension in crayfish muscle fibres.

Zusammenfassung. An isolierten Muskelfasern von *Astacus fluviatilis* wurde mittels Kaliumkontraktur die Spannung-Sarkomerlängenrelation untersucht. Maximale Faserspannung wird bei einer Sarkomerlänge von 10,5 μ entwickelt. Werden Fasern zu einer Sarkomerlänge von 16,5 μ gedehnt, so ist die Spannung nicht mehr messbar. Diese Sarkomerlänge ist etwas grösser als diejenige (14,45 μ), die aus der Länge der dicken Filamente (3,95 μ) und der optimalen Länge hervorgeht.

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⁷ F. CARLSEN, G. G. KNAPPEIS, and F. BUCHTHAL, *J. biophys. biochem. Cytol.* 11, 95 (1961).

⁸ A. M. GORDON, A. F. HUXLEY, and F. J. JULIAN, *J. Physiol.* 167, 42 P (1963).

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Occurrence and Morphology of a Fibrous Body in the Mitochondria of the Slime Mold *Physarum polycephalum*¹

In a previous paper² we have reported that the mitochondria of the slime mold, *Physarum polycephalum*, when incubated with tritiated thymidine (10 μ Ci/ml) incorporate this DNA precursor to such an extent that an intensive autoradiographic picture can be obtained by conventional procedures. In a search for a structural component of the mitochondria that might be related to this unusually high uptake of a DNA precursor, we found that the mitochondria contain a previously unknown fibrous body of considerable size. This body was present after different fixations, e.g. 2% osmic acid in growth medium³ (pH 5), 1% osmic acid in collidine buffer (pH 7.8), and Palade's fixative. The latter was employed in obtaining the following electronmicrographs.

Surface plasmodia were prepared from microplasmodia as described previously⁴. Small explants from these plasmodia were fixed for 1 h in glutaraldehyde (2%, phosphate buffer, pH 7.4) and then placed, for 2 h, in Palade's fixative (1% osmic acid in veronal buffer, pH 7.4). The pieces were embedded in Epon according to LUFT⁵. Silver sections were prepared with a Porter-

¹ Supported by grants from USPHS (Grant No. GM 11949-03 and HD-0052-05).

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⁴ E. GUTTES and S. GUTTES, in *Methods in Cell Physiology* (Ed., D. M. PRESCOTT; Academic Press Inc., New York 1964), vol. 1, p. 43.

⁵ J. LUFT, *J. biophys. biochem. Cytol.* 9, 409 (1961).